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(54) Title: COMPOSITIONS AND METHODS FOR THE THERAPY AND DIAGNOSIS OF COLON CANCER

(57) Abstract: Compositions and methods for the therapy and diagnosis of cancer, such as colon cancer, are disclosed. Compositions may comprise one or more colon tumor proteins, immunogenic portions thereof, or polynucleotides that encode such portions. Alternatively, a therapeutic composition may comprise an antigen presenting cell that expresses a colon tumor protein, or a T cell that is specific for cells expressing such a protein. Such compositions may be used, for example, for the prevention and treatment of diseases such as colon cancer. Diagnostic methods based on detecting a colon tumor protein, or mRNA encoding such a protein, in a sample are also provided.

COMPOSITIONS AND METHODS FOR THE THERAPY AND DIAGNOSIS OF COLON CANCER

TECHNICAL FIELD OF THE INVENTION

The present invention relates generally to therapy and diagnosis of cancer, such as colon cancer. The invention is more specifically related to polypeptides comprising at least a portion of a colon tumor protein, and to polynucleotides encoding such polypeptides. Such polypeptides and polynucleotides may be used in vaccines and pharmaceutical compositions for prevention and treatment of colon malignancies, and for the diagnosis and monitoring of such cancers.

BACKGROUND OF THE INVENTION

Cancer is a significant health problem throughout the world. Although advances have been made in detection and therapy of cancer, no vaccine or other universally successful method for prevention or treatment is currently available. Current therapies, which are generally based on a combination of chemotherapy or surgery and radiation, continue to prove inadequate in many patients.

Colon cancer is the second most frequently diagnosed malignancy in the United States as well as the second most common cause of cancer death. The five-year survival rate for patients with colorectal cancer detected in an early localized stage is 92%; unfortunately, only 37% of colorectal cancer is diagnosed at this stage. The survival rate drops to 64% if the cancer is allowed to spread to adjacent organs or lymph nodes, and to 7% in patients with distant metastases.

The prognosis of colon cancer is directly related to the degree of penetration of the tumor through the bowel wall and the presence or absence of nodal involvement, consequently early detection and treatment are especially important. Currently, diagnosis is aided by the use of screening assays for fecal occult blood, sigmoidoscopy, colonoscopy and double contrast barium enemas. Treatment regimens are determined by the type and stage of the cancer, and include surgery, radiation therapy and/or chemotherapy. Recurrence following surgery (the most common form of therapy) is a major problem and is often the ultimate cause of death.

In spite of considerable research into therapies for these and other cancers, colon cancer remains difficult to diagnose and treat effectively. Accordingly, there is a need in the art for improved methods for detecting and treating such cancers. The present invention fulfills these needs and further provides other related advantages.

SUMMARY OF THE INVENTION

In one aspect, the present invention provides polynucleotide compositions comprising a sequence selected from the group consisting of:

(a) sequences provided in SEQ ID NOs:1-234, 236, and 244;

(b) complements of the sequences provided in SEQ ID NOs:1-234, 236, and 244;

(c) sequences consisting of at least 20, 25, 30, 35, 40, 45, 50, 75 and 100 contiguous residues of a sequence provided in SEQ ID NOs:1-234, 236, and 244;

(d) sequences that hybridize to a sequence provided in SEQ ID NOs:1-234, 236, and 244, under moderate or highly stringent conditions;

(e) sequences having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to a sequence of SEQ ID NOs:1-234, 236, and 244;

(f) degenerate variants of a sequence provided in SEQ ID NOs:1-234, 236, and 244.

In one preferred embodiment, the polynucleotide compositions of the invention are expressed in at least about 20%, more preferably in at least about 30%, and most preferably in at least about 50% of colon tumor samples tested, at a level that is at least about 2-fold, preferably at least about 5-fold, and most preferably at least about 10-fold higher than that for normal tissues.

The present invention, in another aspect, provides polypeptide compositions comprising an amino acid sequence that is encoded by a polynucleotide sequence described above.

The present invention further provides polypeptide compositions comprising an amino acid sequence selected from the group consisting of sequences recited in SEQ ID NOs:235, 237, and 245.

In certain preferred embodiments, the polypeptides and/or polynucleotides of the present invention are immunogenic, *i.e.*, they are capable of eliciting an immune response, particularly a humoral and/or cellular immune response, as further described herein.

The present invention further provides fragments, variants and/or derivatives of the disclosed polypeptide and/or polynucleotide sequences, wherein the fragments, variants and/or derivatives preferably have a level of immunogenic activity of at least about 50%, preferably at least about 70% and more preferably at least about 90% of the level of immunogenic activity of a polypeptide sequence set forth in SEQ ID NOs:235, 237, and 245 or a polypeptide sequence encoded by a polynucleotide sequence set forth in SEQ ID NOs:1-234, 236, and 244.

The present invention further provides polynucleotides that encode a polypeptide described above, expression vectors comprising such polynucleotides and host cells transformed or transfected with such expression vectors.

Within other aspects, the present invention provides pharmaceutical compositions comprising a polypeptide or polynucleotide as described above and a physiologically acceptable carrier.

Within a related aspect of the present invention, the pharmaceutical compositions, *e.g.*, vaccine compositions, are provided for prophylactic or therapeutic applications. Such compositions generally comprise an immunogenic polypeptide or polynucleotide of the invention and an immunostimulant, such as an adjuvant.

The present invention further provides pharmaceutical compositions that comprise: (a) an antibody or antigen-binding fragment thereof that specifically binds to a polypeptide of the present invention, or a fragment thereof; and (b) a physiologically acceptable carrier.

Within further aspects, the present invention provides pharmaceutical compositions comprising: (a) an antigen presenting cell that expresses a polypeptide as described above and (b) a pharmaceutically acceptable carrier or excipient. Illustrative antigen presenting cells include dendritic cells, macrophages, monocytes, fibroblasts and B cells.

Within related aspects, pharmaceutical compositions are provided that comprise: (a) an antigen presenting cell that expresses a polypeptide as described above and (b) an immunostimulant.

The present invention further provides, in other aspects, fusion proteins that comprise at least one polypeptide as described above, as well as polynucleotides encoding such fusion proteins, typically in the form of pharmaceutical compositions, *e.g.*, vaccine compositions, comprising a physiologically acceptable carrier and/or an immunostimulant. The fusions proteins may comprise multiple immunogenic polypeptides or portions/variants thereof, as described herein, and may further comprise one or more polypeptide segments for facilitating the expression, purification and/or immunogenicity of the polypeptide(s).

Within further aspects, the present invention provides methods for stimulating an immune response in a patient, preferably a T cell response in a human patient, comprising administering a pharmaceutical composition described herein. The patient may be afflicted with colon cancer, in which case the methods provide treatment for the disease, or patient considered at risk for such a disease may be treated prophylactically.

Within further aspects, the present invention provides methods for inhibiting the development of a cancer in a patient, comprising administering to a patient a pharmaceutical composition as recited above. The patient may be afflicted with colon cancer, in which case the methods provide treatment for the disease, or patient considered at risk for such a disease may be treated prophylactically.

The present invention further provides, within other aspects, methods for removing tumor cells from a biological sample, comprising contacting a biological sample with T cells that specifically react with a polypeptide of the present invention, wherein the step of contacting is performed under conditions and for a time sufficient to permit the removal of cells expressing the protein from the sample.

Within related aspects, methods are provided for inhibiting the development of a cancer in a patient, comprising administering to a patient a biological sample treated as described above.

Methods are further provided, within other aspects, for stimulating and/or expanding T cells specific for a polypeptide of the present invention, comprising contacting T cells with one or more of: (i) a polypeptide as described above; (ii) a polynucleotide encoding such a polypeptide; and/or (iii) an antigen presenting cell that expresses such a polypeptide; under conditions and for a time sufficient to permit the stimulation and/or expansion of T cells. Isolated T cell populations comprising T cells prepared as described above are also provided.

Within further aspects, the present invention provides methods for inhibiting the development of a cancer in a patient, comprising administering to a patient an effective amount of a T cell population as described above.

The present invention further provides methods for inhibiting the development of a cancer in a patient, comprising the steps of: (a) incubating $CD4^+$ and/or $CD8^+$ T cells isolated from a patient with one or more of: (i) a polypeptide comprising at least an immunogenic portion of polypeptide disclosed herein; (ii) a polynucleotide encoding such a polypeptide; and (iii) an antigen-presenting cell that expressed such a polypeptide; and (b) administering to the patient an effective amount of the proliferated T cells, and thereby inhibiting the development of a cancer in the patient. Proliferated cells may, but need not, be cloned prior to administration to the patient.

Within further aspects, the present invention provides methods for determining the presence or absence of a cancer, preferably a colon cancer, in a patient comprising: (a) contacting a biological sample obtained from a patient with a binding agent that binds to a polypeptide as recited above; (b) detecting in the sample an amount of polypeptide that binds to the binding agent; and (c) comparing the amount of polypeptide with a predetermined cut-off value, and therefrom determining the presence or absence of a cancer in the patient. Within preferred embodiments, the binding agent is an antibody, more preferably a monoclonal antibody.

The present invention also provides, within other aspects, methods for monitoring the progression of a cancer in a patient. Such methods comprise the steps of: (a) contacting a biological sample obtained from a patient at a first point in time with a binding agent that binds to a polypeptide as recited above; (b) detecting in the

sample an amount of polypeptide that binds to the binding agent; (c) repeating steps (a) and (b) using a biological sample obtained from the patient at a subsequent point in time; and (d) comparing the amount of polypeptide detected in step (c) with the amount detected in step (b) and therefrom monitoring the progression of the cancer in the patient.

The present invention further provides, within other aspects, methods for determining the presence or absence of a cancer in a patient, comprising the steps of: (a) contacting a biological sample, e.g., tumor sample, serum sample, etc., obtained from a patient with an oligonucleotide that hybridizes to a polynucleotide that encodes a polypeptide of the present invention; (b) detecting in the sample a level of a polynucleotide, preferably mRNA, that hybridizes to the oligonucleotide; and (c) comparing the level of polynucleotide that hybridizes to the oligonucleotide with a predetermined cut-off value, and therefrom determining the presence or absence of a cancer in the patient. Within certain embodiments, the amount of mRNA is detected via polymerase chain reaction using, for example, at least one oligonucleotide primer that hybridizes to a polynucleotide encoding a polypeptide as recited above, or a complement of such a polynucleotide. Within other embodiments, the amount of mRNA is detected using a hybridization technique, employing an oligonucleotide probe that hybridizes to a polynucleotide that encodes a polypeptide as recited above, or a complement of such a polynucleotide.

In related aspects, methods are provided for monitoring the progression of a cancer in a patient, comprising the steps of: (a) contacting a biological sample obtained from a patient with an oligonucleotide that hybridizes to a polynucleotide that encodes a polypeptide of the present invention; (b) detecting in the sample an amount of a polynucleotide that hybridizes to the oligonucleotide; (c) repeating steps (a) and (b) using a biological sample obtained from the patient at a subsequent point in time; and (d) comparing the amount of polynucleotide detected in step (c) with the amount detected in step (b) and therefrom monitoring the progression of the cancer in the patient.

Within further aspects, the present invention provides antibodies, such as monoclonal antibodies, that bind to a polypeptide as described above, as well as

diagnostic kits comprising such antibodies. Diagnostic kits comprising one or more oligonucleotide probes or primers as described above are also provided.

These and other aspects of the present invention will become apparent upon reference to the following detailed description. All references disclosed herein are hereby incorporated by reference in their entirety as if each was incorporated individually.

BRIEF DESCRIPTION OF THE SEQUENCE IDENTIFIERS

SEQ ID NO: 1 is the determined cDNA sequence for 54172.1.

SEQ ID NO: 2 is the determined cDNA sequence for 54104.1 which shares homology with PAC 75N13 on chromosome Xq21.1.

SEQ ID NO: 3 is the determined cDNA sequence for 53978.1 which shares homology with Glutamine:fructose-6 phosphate amidotransferase.

SEQ ID NO: 4 is the determined cDNA sequence for 54184.1 which shares homology with Colon Kruppel-like factor.

SEQ ID NO: 5 is the determined cDNA sequence for 54149.1 which shares homology with cDNA FLJ10461 fis, clone NT2RP1001482.

SEQ ID NO: 6 is the determined cDNA sequence for 54034.1.

SEQ ID NO: 7 is the determined cDNA sequence for 54085.1 which shares homology with Human beta 2 gene.

SEQ ID NO: 8 is the determined cDNA sequence for 53948.1 which shares homology with 12p12 BAC RPCl11-267J23.

SEQ ID NO: 9 is the determined cDNA sequence for 54026.1 which shares homology with Clone 164F3 on chromosome X2q21.33-23.

SEQ ID NO: 10 is the determined cDNA sequence for 53907.1 which shares homology with Lysyl hydroxylase isoform 2.

SEQ ID NO: 11 is the determined cDNA sequence for 54066.1 which shares homology with Mucin 11.

SEQ ID NO: 12 is the determined cDNA sequence for 54017.1 which shares homology with Mucin 11.

SEQ ID NO: 13 is the determined cDNA sequence for 54006.1 which shares homology with Mucin 11.

SEQ ID NO: 14 is the determined cDNA sequence for 53962.1 which shares homology with Epiregulin (EGF family).

SEQ ID NO: 15 is the determined cDNA sequence for 54028.1 which shares homology with Mucin 12.

SEQ ID NO: 16 is the determined cDNA sequence for 54166.1 which shares homology with E1A enhancer binding protein.

SEQ ID NO: 17 is the determined cDNA sequence for 54174.1 which shares homology with PAC clone RP1-170O19 from 7p15-p21.

SEQ ID NO: 18 is the determined cDNA sequence for 53949.1.

SEQ ID NO: 19 is the determined cDNA sequence for 53898.1.

SEQ ID NO: 20 is the determined cDNA sequence for 54069.1.

SEQ ID NO: 21 is the determined cDNA sequence for 54048.1 which shares homology with cDNA FLJ20676 fis, clone KAIA4294.

SEQ ID NO: 22 is the determined cDNA sequence for 54031.1 which shares homology with Chromosome 17, clone hRPC.1171_I_10.

SEQ ID NO: 23 is the determined cDNA sequence for 54154.1 which shares homology with Alpha topoisomerase truncated form.

SEQ ID NO: 24 is the determined cDNA sequence for 54009.1 which shares homology with Cytokeratin 20.

SEQ ID NO: 25 is the determined cDNA sequence for 54070.1 which shares homology with Erythroblastosis virus oncogene homolog 2.

SEQ ID NO: 26 is the determined cDNA sequence for 53998.1 which shares homology with Polyadenylate binding protein II.

SEQ ID NO: 27 is the determined cDNA sequence for 54089.1.

SEQ ID NO: 28 is the determined cDNA sequence for 54182.1 which shares homology with Transforming growth factor-beta induced gene product.

SEQ ID NO: 29 is the determined cDNA sequence for 53989.1 which shares homology with GDP-mannose 4,6 dehydratase.

SEQ ID NO: 30 is the determined cDNA sequence for 54181.1.

SEQ ID NO: 31 is the determined cDNA sequence for 54079.1 which shares homology with PAC 75N13 on chromosome Xq21.1.

SEQ ID NO: 32 is the determined cDNA sequence for 54114.1 which shares homology with Mus fork head transcription factor gene.

SEQ ID NO: 33 is the determined cDNA sequence for 54160.1 which shares homology with Clone 146H21 on chromosome Xq22.

SEQ ID NO: 34 is the determined cDNA sequence for 54168.1 which shares homology with Glutamine:fructose-6-phosphate amidotransferase.

SEQ ID NO: 35 is the determined cDNA sequence for 54078.1 which shares homology with PAC 75N13 on chromosome Xq21.1.

SEQ ID NO: 36 is the determined cDNA sequence for 53900.1 which shares homology with Intestinal peptide-associated transporter HPT-1.

SEQ ID NO: 37 is the determined cDNA sequence for 54147.1.

SEQ ID NO: 38 is the determined cDNA sequence for 54033.1 which shares homology with Human proteinase activated receptor-2.

SEQ ID NO: 39 is the determined cDNA sequence for 53908.1 which shares homology with GalNAc-T3 gene.

SEQ ID NO: 40 is the determined cDNA sequence for 54022.1.

SEQ ID NO: 41 is the determined cDNA sequence for 54039.1 which shares homology with Constitutive fragile sequence.

SEQ ID NO: 42 is the determined cDNA sequence for 54037.1 which shares homology with CD24 signal transducer gene.

SEQ ID NO: 43 is the determined cDNA sequence for 54129.1 which shares homology with Human c-myb gene.

SEQ ID NO: 44 is the determined cDNA sequence for 54054.1 which shares homology with Pyrroline-t-carboxylate synthase long form.

SEQ ID NO: 45 is the determined cDNA sequence for 54055.1 which shares homology with Human zinc finger protein ZNF-139.

SEQ ID NO: 46 is the determined cDNA sequence for 54046.1 which shares homology with Gene for membrane cofactor protein.

SEQ ID NO: 47 is the determined cDNA sequence for 54047.1 which shares homology with Colon Kruppel-like factor.

SEQ ID NO: 48 is the determined cDNA sequence for 54040.1 which shares homology with Human capping protein alpha subunit isoform 1.

SEQ ID NO: 49 is the determined cDNA sequence for 54035.1 which shares homology with Ig lambda-chain.

SEQ ID NO: 50 is the determined cDNA sequence for 54130.1 which shares homology with Protein tyrosine kinase.

SEQ ID NO: 51 is the determined cDNA sequence for 54045.1 which shares homology with cDNA FLJ10610 fis, clone NT2RP2005293.

SEQ ID NO: 52 is the determined cDNA sequence for 54052.1 which shares homology with Human microtubule-associated protein 7.

SEQ ID NO: 53 is the determined cDNA sequence for 54050.1 which shares homology with Human retinoblastoma susceptibility protein.

SEQ ID NO: 54 is the determined cDNA sequence for 54051.1 which shares homology with Human reticulocalbin.

SEQ ID NO: 55 is the determined cDNA sequence for 54178.1 which shares homology with Translation initiation factor e1F3 p36 subunit.

SEQ ID NO: 56 is the determined cDNA sequence for 54148.1 which shares homology with Human apurinic/apyrimidinic-endonuclease.

SEQ ID NO: 57 is the determined cDNA sequence for 54058.1.

SEQ ID NO: 58 is the determined cDNA sequence for 54059.1 which shares homology with Human integral transmembrane protein 1.

SEQ ID NO: 59 is the determined cDNA sequence for 54126.1 which shares homology with Human serine kinase.

SEQ ID NO: 60 is the determined cDNA sequence for 54127.1 which shares homology with Human CG1-44 protein.

SEQ ID NO: 61 is the determined cDNA sequence for 54049.1 which shares homology with HADH/NADPH thyroid oxidase p138-tox protein.

SEQ ID NO: 62 is the determined cDNA sequence for 54056.1 which shares homology with Human peptide transporter (TAP1) protein.

SEQ ID NO: 63 is the determined cDNA sequence for 54064.1 which shares homology with Clone RP1-39G22 on chromosome 1p32.1-34.3.

SEQ ID NO: 64 is the determined cDNA sequence for 54124.1 which shares homology with Clone Transforming growth factor-beta induced gene product.

SEQ ID NO: 65 is the determined cDNA sequence for 54063.1.

SEQ ID NO: 66 is the determined cDNA sequence for 54141.1 which shares homology with Cytokeratin 8.

SEQ ID NO: 67 is the determined cDNA sequence for 54119.1 which shares homology with Human coat protein gamma-cop.

SEQ ID NO: 68 is the determined cDNA sequence for 54111.1 which shares homology with Bumetanide-sensitive Na-K-Cl cotransporter.

SEQ ID NO: 69 is the determined cDNA sequence for 54121.1 which shares homology with cDNA FLJ10969 fis, clone PLACE1000909.

SEQ ID NO: 70 is the determined cDNA sequence for 54065.1 which shares homology with BAC clone 215O12.

SEQ ID NO: 71 is the determined cDNA sequence for 54060.1 which shares homology with Autoantigen calreticulin.

SEQ ID NO: 72 is the determined cDNA sequence for 54125.1 which shares homology with Human hepatic squalene synthetase.

SEQ ID NO: 73 is the determined cDNA sequence for 54143.1 which shares homology with Human RAD21 homolog.

SEQ ID NO: 74 is the determined cDNA sequence for 54139.1 which shares homology with Human MHC class II HLA-DR-alpha.

SEQ ID NO: 75 is the determined cDNA sequence for 54137.1 which shares homology with Human Claudin-7.

SEQ ID NO: 76 is the determined cDNA sequence for 54044.1 which shares homology with Ribosome protein S6 kinase 1.

SEQ ID NO: 77 is the determined cDNA sequence for 54042.1 which shares homology with CO-029 tumor associated antigen.

SEQ ID NO: 78 is the determined cDNA sequence for 54043.1 which shares homology with KIAA1077 protein.

SEQ ID NO: 79 is the determined cDNA sequence for 54136.1 which shares homology with Human lipocortin II.

SEQ ID NO: 80 is the determined cDNA sequence for 54157.1 which shares homology with PAC 454G6 on chromosome 1q24.

SEQ ID NO: 81 is the determined cDNA sequence for 54140.1.

SEQ ID NO: 82 is the determined cDNA sequence for 54120.1.

SEQ ID NO: 83 is the determined cDNA sequence for 54145.1 which shares homology with KIAA0152.

SEQ ID NO: 84 is the determined cDNA sequence for 54117.1 which shares homology with Tumor antigen L6.

SEQ ID NO: 85 is the determined cDNA sequence for 54116.1 which shares homology with UDP-N-acetylglucosamine transporter.

SEQ ID NO: 86 is the determined cDNA sequence for 54151.1.

SEQ ID NO: 87 is the determined cDNA sequence for 54152.1 which shares homology with Cystine/glutamate transporter.

SEQ ID NO: 88 is the determined cDNA sequence for 54115.1.

SEQ ID NO: 89 is the determined cDNA sequence for 54146.1 which shares homology with GAPDH.

SEQ ID NO: 90 is the determined cDNA sequence for 54155.1 which shares homology with cDNA DKFZp586O0118.

SEQ ID NO: 91 is the determined cDNA sequence for 54159.1.

SEQ ID NO: 92 is the determined cDNA sequence for 54020.1 which shares homology with Neutrophil lipocalin.

SEQ ID NO: 93 is the determined cDNA sequence for 54169.1 which shares homology with Nuclear matrix protein NRP/B.

SEQ ID NO: 94 is the determined cDNA sequence for 54167.1 which shares homology with CGI-151/KIAA0992 protein.

SEQ ID NO: 95 is the determined cDNA sequence for 54030.1.

SEQ ID NO: 96 is the determined cDNA sequence for 54161.1.

SEQ ID NO: 97 is the determined cDNA sequence for 54162.1 which shares homology with Poly A binding protein.

SEQ ID NO: 98 is the determined cDNA sequence for 54163.1 which shares homology with Ribosome protein L13.

SEQ ID NO: 99 is the determined cDNA sequence for 54164.1 which shares homology with Human alpha enolase.

SEQ ID NO: 100 is the determined cDNA sequence for 54132.1 which shares homology with Human E-1 enzyme.

SEQ ID NO: 101 is the determined cDNA sequence for 54112.1 which shares homology with cDNA DKFZp58612022.

SEQ ID NO: 102 is the determined cDNA sequence for 54133.1 which shares homology with Human ZW10 interactor Zwint.

SEQ ID NO: 103 is the determined cDNA sequence for 54165.1 which shares homology with Bumetanide-sensitive Na-K-Cl cotransporter.

SEQ ID NO: 104 is the determined cDNA sequence for 54158.1 which shares homology with cDNA FLJ10549 fis, clone NT2RP2001976.

SEQ ID NO: 105 is the determined cDNA sequence for 54131.1 which shares homology with cDNA DKFZp434C0523.

SEQ ID NO: 106 is the determined cDNA sequence for 54122.1.

SEQ ID NO: 107 is the determined cDNA sequence for 54098.1.

SEQ ID NO: 108 is the determined cDNA sequence for 54173.1 which shares homology with NADH-ubiquinone oxidoreductase NDUFS2 subunit.

SEQ ID NO: 109 is the determined cDNA sequence for 54108.1 which shares homology with Phospholipase A2.

SEQ ID NO: 110 is the determined cDNA sequence for 54175.1 which shares homology with cDNA FLJ10610 fis, clone NT2RP2005293.

SEQ ID NO: 111 is the determined cDNA sequence for 54179.1 which shares homology with Ig heavy chain variable region.

SEQ ID NO: 112 is the determined cDNA sequence for 54177.1 which shares homology with Protein phosphatase 2C gamma.

SEQ ID NO: 113 is the determined cDNA sequence for 54170.1 which shares homology with Cyclin protein.

SEQ ID NO: 114 is the determined cDNA sequence for 54176.1 which shares homology with Transgelin 2 (predicted).

SEQ ID NO: 115 is the determined cDNA sequence for 54180.1 which shares homology with Human GalNAc-T3 gene.

SEQ ID NO: 116 is the determined cDNA sequence for 53897.1 which shares homology with cDNA FLJ10884 fis, clone NT2RP4001950.

SEQ ID NO: 117 is the determined cDNA sequence for 54027.1.

SEQ ID NO: 118 is the determined cDNA sequence for 54183.1 which shares homology with Alpha topoisomerase truncated form.

SEQ ID NO: 119 is the determined cDNA sequence for 54107.1 which shares homology with KIAA 1289.

SEQ ID NO: 120 is the determined cDNA sequence for 54106.1 which shares homology with AD022 protein.

SEQ ID NO: 121 is the determined cDNA sequence for 53902.1.

SEQ ID NO: 122 is the determined cDNA sequence for 53918.1 which shares homology with Chromosome 17, clone hRPK.692_E_18.

SEQ ID NO: 123 is the determined cDNA sequence for 53904.1.

SEQ ID NO: 124 is the determined cDNA sequence for 53910.1 which shares homology with cDNA FLJ10823 fis, clone NT2RP4001080.

SEQ ID NO: 125 is the determined cDNA sequence for 53903.1 which shares homology with Vector.

SEQ ID NO: 126 is the determined cDNA sequence for 54103.1.

SEQ ID NO: 127 is the determined cDNA sequence for 53917.1 which shares homology with Cytochrome P450 IIIA4.

SEQ ID NO: 128 is the determined cDNA sequence for 54004.1 which shares homology with CEA.

SEQ ID NO: 129 is the determined cDNA sequence for 53913.1 which shares homology with Protein phosphatase (KAP1).

SEQ ID NO: 130 is the determined cDNA sequence for 54134.1.

SEQ ID NO: 131 is the determined cDNA sequence for 53999.1 which shares homology with Alpha enolase.

SEQ ID NO: 132 is the determined cDNA sequence for 53938.1 which shares homology with Histone deacetylase HD1.

SEQ ID NO: 133 is the determined cDNA sequence for 53939.1 which shares homology with citb_338_f_24, complete sequence.

SEQ ID NO: 134 is the determined cDNA sequence for 53928.1 which shares homology with Human squalene epoxidase.

SEQ ID NO: 135 is the determined cDNA sequence for 53914.1 which shares homology with Human aspartyl-tRNA-synthetase alpha-2 subunit.

SEQ ID NO: 136 is the determined cDNA sequence for 53915.1 which shares homology with Gamma-actin.

SEQ ID NO: 137 is the determined cDNA sequence for 54101.1 which shares homology with Human AP-mu chain family member mu1B.

SEQ ID NO: 138 is the determined cDNA sequence for 53922.1 which shares homology with Human Cctg mRNA for chaperonin.

SEQ ID NO: 139 is the determined cDNA sequence for 54023.1 which shares homology with Chromosome 19.

SEQ ID NO: 140 is the determined cDNA sequence for 53930.1 which shares homology with Human MEGF7.

SEQ ID NO: 141 is the determined cDNA sequence for 53921.1 which shares homology with Connexin 26.

SEQ ID NO: 142 is the determined cDNA sequence for 54002.1 which shares homology with Human dipeptidyl peptidase IV.

SEQ ID NO: 143 is the determined cDNA sequence for 54003.1 which shares homology with Chromosome 5 clone CTC-436P18.

SEQ ID NO: 144 is the determined cDNA sequence for 54005.1 which shares homology with Human 2-oxoglutarate dehydrogenase.

SEQ ID NO: 145 is the determined cDNA sequence for 53925.1 which shares homology with RHO guanine nucleotide-exchange factor.

SEQ ID NO: 146 is the determined cDNA sequence for 53927.1 which shares homology with 12q24 PAC RPCI1-261P5.

SEQ ID NO: 147 is the determined cDNA sequence for 54083.1 which shares homology with Human colon mucosa-associated mRNA.

SEQ ID NO: 148 is the determined cDNA sequence for 53937.1.

SEQ ID NO: 149 is the determined cDNA sequence for 54074.1 which shares homology with Clone RP4-621F18 on chromosome 1p11.4-21.3.

SEQ ID NO: 150 is the determined cDNA sequence for 54105.1.

SEQ ID NO: 151 is the determined cDNA sequence for 53961.1 which shares homology with Human embryonic lung protein.

SEQ ID NO: 152 is the determined cDNA sequence for 53919.1.

SEQ ID NO: 153 is the determined cDNA sequence for 53933.1 which shares homology with Human leukocyte surface protein CD31.

SEQ ID NO: 154 is the determined cDNA sequence for 53972.1 which shares homology with cDNA FLJ10679 fis, clone NT2RP2006565.

SEQ ID NO: 155 is the determined cDNA sequence for 53906.1.

SEQ ID NO: 156 is the determined cDNA sequence for 53924.1 which shares homology with Poly A binding protein.

SEQ ID NO: 157 is the determined cDNA sequence for 54144.1.

SEQ ID NO: 158 is the determined cDNA sequence for 54068.1 which shares homology with Cystic fibrosis transmembrane conductance regulator.

SEQ ID NO: 159 is the determined cDNA sequence for 53929.1.

SEQ ID NO: 160 is the determined cDNA sequence for 53959.1 which shares homology with KIAA1050.

SEQ ID NO: 161 is the determined cDNA sequence for 53942.1.

SEQ ID NO: 162 is the determined cDNA sequence for 53931.1 which shares homology with cDNA FLJ11127 fis, clone PLACE 1006225.

SEQ ID NO: 163 is the determined cDNA sequence for 53935.1 which shares homology with Human set gene.

SEQ ID NO: 164 is the determined cDNA sequence for 54099.1 which shares homology with Human pleckstrin 2.

SEQ ID NO: 165 is the determined cDNA sequence for 53943.1 which shares homology with KIAA0965.

SEQ ID NO: 166 is the determined cDNA sequence for 54000.1 which shares homology with Tis 11d gene.

SEQ ID NO: 167 is the determined cDNA sequence for 54100.1 which shares homology with Cyhtokine (GRO-gamma).

SEQ ID NO: 168 is the determined cDNA sequence for 53940.1 which shares homology with Human p85Mcm mRNA.

SEQ ID NO: 169 is the determined cDNA sequence for 53941.1 which shares homology with cDNA DKFZp586H0519.

SEQ ID NO: 170 is the determined cDNA sequence for 53953.1 which shares homology with SOX9.

SEQ ID NO: 171 is the determined cDNA sequence for 54007.1 which shares homology with VAV-like protein.

SEQ ID NO: 172 is the determined cDNA sequence for 53950.1 which shares homology with NF-E2 related factor 3.

SEQ ID NO: 173 is the determined cDNA sequence for 53968.1 which shares homology with cDNA FLJ20127 fis, clone COL06176.

SEQ ID NO: 174 is the determined cDNA sequence for 53945.1.

SEQ ID NO: 175 is the determined cDNA sequence for 54091.1.

SEQ ID NO: 176 is the determined cDNA sequence for 54013.1 which shares homology with Human argininosuccinate synthetase.

SEQ ID NO: 177 is the determined cDNA sequence for 54092.1 which shares homology with Human serine kinase.

SEQ ID NO: 178 is the determined cDNA sequence for 54095.1 which shares homology with Clone RP1-155G6 on chromosome 20.

SEQ ID NO: 179 is the determined cDNA sequence for 53987.1 which shares homology with Human phospholipase C beta 4.

SEQ ID NO: 180 is the determined cDNA sequence for 53967.1.

SEQ ID NO: 181 is the determined cDNA sequence for 53963.1 which shares homology with VAV-3 protein.

SEQ ID NO: 182 is the determined cDNA sequence for 54032.1.

SEQ ID NO: 183 is the determined cDNA sequence for 54067.1 which shares homology with PAC RPCI-1 133G21 map 21q11.1 region D21S190.

SEQ ID NO: 184 is the determined cDNA sequence for 54057.1 which shares homology with Calcium-binding protein S100P.

SEQ ID NO: 185 is the determined cDNA sequence for 54135.1 which shares homology with Human leupaxin.

SEQ ID NO: 186 is the determined cDNA sequence for 53969.1 which shares homology with VAV-3 Protein.

SEQ ID NO: 187 is the determined cDNA sequence for 53970.1.

SEQ ID NO: 188 is the determined cDNA sequence for 53966.1 which shares homology with hnRNP type A/B protein.

SEQ ID NO: 189 is the determined cDNA sequence for 53995.1 which shares homology with Human cell cycle control gene CDC2.

SEQ ID NO: 190 is the determined cDNA sequence for 54075.1.

SEQ ID NO: 191 is the determined cDNA sequence for 54094.1.

SEQ ID NO: 192 is the determined cDNA sequence for 53977.1.

SEQ ID NO: 193 is the determined cDNA sequence for 54123.1 which shares homology with BAC clone RG083M05 from 7q21-7q22.

SEQ ID NO: 194 is the determined cDNA sequence for 53960.1 which shares homology with Human STS WI-14644.

SEQ ID NO: 195 is the determined cDNA sequence for 53976.1 which shares homology with Human glutaminyl-tRNA synthetase.

SEQ ID NO: 196 is the determined cDNA sequence for 54096.1 which shares homology with Human 26S proteasome-associated pad 1 homolog.

SEQ ID NO: 197 is the determined cDNA sequence for 54110.1 which shares homology with Human squalene epoxidase.

SEQ ID NO: 198 is the determined cDNA sequence for 53920.1 which shares homology with Human nuclear chloride ion channel protein.

SEQ ID NO: 199 is the determined cDNA sequence for 53979.1 which shares homology with PAC RPCI-1 133G21 map 21q11.1 region D21S190.

SEQ ID NO: 200 is the determined cDNA sequence for 54081.1 which shares homology with PAC clone RP5-1185I7 from 7q11.23-q21.

SEQ ID NO: 201 is the determined cDNA sequence for 54082.1 which shares homology with Human ephrin.

SEQ ID NO: 202 is the determined cDNA sequence for 53986.1 which shares homology with cDNA FLJ20673 fis, clone KAIA4464.

SEQ ID NO: 203 is the determined cDNA sequence for 53992.1.

SEQ ID NO: 204 is the determined cDNA sequence for 54016.1.

SEQ ID NO: 205 is the determined cDNA sequence for 54018.1 which shares homology with CD9 antigen.

SEQ ID NO: 206 is the determined cDNA sequence for 53985.1 which shares homology with KIAA0715.

SEQ ID NO: 207 is the determined cDNA sequence for 53973.1 which shares homology with Cyclin B.

SEQ ID NO: 208 is the determined cDNA sequence for 54012.1 which shares homology with KIAA1225.

SEQ ID NO: 209 is the determined cDNA sequence for 53982.1.

SEQ ID NO: 210 is the determined cDNA sequence for 53988.1 which shares homology with Colon mucosa-associated mRNA.

SEQ ID NO: 211 is the determined cDNA sequence for 53990.1 which shares homology with cDNA FLJ20171 fis, clone COL09761.

SEQ ID NO: 212 is the determined cDNA sequence for 53991.1.

SEQ ID NO: 213 is the determined cDNA sequence for 51519.1 which shares homology with CEA.

SEQ ID NO: 214 is the determined cDNA sequence for 51507.1 which shares homology with Adenocarcinoma-associated antigen.

SEQ ID NO: 215 is the determined cDNA sequence for 51435.1 which shares homology with Secreted protein XAG.

SEQ ID NO: 216 is the determined cDNA sequence for 51425.1 which shares homology with Adenocarcinoma-associated antigen.

SEQ ID NO: 217 is the determined cDNA sequence for 51548.1.

SEQ ID NO: 218 is the determined cDNA sequence for 51430.1 which shares homology with CEA.

SEQ ID NO: 219 is the determined cDNA sequence for 51549.1 which shares homology with CEA.

SEQ ID NO: 220 is the determined cDNA sequence for 51439.1 which shares homology with Nonspecific crossreacting antigen.

SEQ ID NO: 221 is the determined cDNA sequence for 51535.1 which shares homology with Neutrophil gelatinase associated lipocalin.

SEQ ID NO: 222 is the determined cDNA sequence for 51486.1 which shares homology with Transformation growth factor-beta induced gene product.

SEQ ID NO: 223 is the determined cDNA sequence for 51479.1 which shares homology with Undetermined origin found 5′ to NCA mRNA.

SEQ ID NO: 224 is the determined cDNA sequence for 51469.1 which shares homology with Galectin-4.

SEQ ID NO: 225 is the determined cDNA sequence for 51470.1 which shares homology with Nonspecific crossreacting antigen.

SEQ ID NO: 226 is the determined cDNA sequence for 51536.1 which shares homology with Secreted protein XAG.

SEQ ID NO: 227 is the determined cDNA sequence for 51483.1 which shares homology with Clone 146H21 on chromosome Xq22.

SEQ ID NO: 228 is the determined cDNA sequence for 51522.1 which shares homology with GAPDH.

SEQ ID NO: 229 is the determined cDNA sequence for 51485.1 which shares homology with Mucin 11.

SEQ ID NO: 230 is the determined cDNA sequence for 51460.1 which shares homology with Nonspecific crossreacting antigen.

SEQ ID NO: 231 is the determined cDNA sequence for 51458.1 which shares homology with KIAA0517 protein.

SEQ ID NO: 232 is the determined cDNA sequence for 51506.1 which shares homology with Surface glycoprotein CD44.

SEQ ID NO: 233 is the determined cDNA sequence for 51440.1 which shares homology with Chromosome 21q22.1, D21S226-AML region.

SEQ ID NO: 234 is the determined cDNA sequence for C907P.

SEQ ID NO: 235 is the amino acid sequence for C907P.

SEQ ID NO: 236 is the determine cDNA sequence for Ra12-C915P-f3.

SEQ ID NO: 237 is the amino acid sequence for Ra12-C915P-f3.

SEQ ID NO: 238 is the nucleotide sequence of the AW154 primer.

SEQ ID NO: 239 is the nucleotide sequence of the AW155 primer.

SEQ ID NO: 240 is the nucleotide sequence of the AW156 primer.

SEQ ID NO: 241 is the nucleotide sequence of the AW157 primer.

SEQ ID NO: 242 is the nucleotide sequence of the AW158 primer.

SEQ ID NO: 243 is the nucleotide sequence of the AW159 primer.

SEQ ID NO: 244 is the determined full-length cDNA sequence of C915P.

SEQ ID NO: 245 is the amino acid sequence encoded by the cDNA sequence set forth in SEQ ID NO:244.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed generally to compositions and their use in the therapy and diagnosis of cancer, particularly colon cancer. As described further below, illustrative compositions of the present invention include, but are not restricted to, polypeptides, particularly immunogenic polypeptides, polynucleotides encoding such polypeptides, antibodies and other binding agents, antigen presenting cells (APCs) and immune system cells (*e.g.*, T cells).

The practice of the present invention will employ, unless indicated specifically to the contrary, conventional methods of virology, immunology, microbiology, molecular biology and recombinant DNA techniques within the skill of the art, many of which are described below for the purpose of illustration. Such techniques are explained fully in the literature. See, *e.g.*, Sambrook, et al. Molecular Cloning: A Laboratory Manual (2nd Edition, 1989); Maniatis et al. Molecular Cloning: A Laboratory Manual (1982); DNA Cloning: A Practical Approach, vol. I & II (D.

Glover, ed.); Oligonucleotide Synthesis (N. Gait, ed., 1984); Nucleic Acid Hybridization (B. Hames & S. Higgins, eds., 1985); Transcription and Translation (B. Hames & S. Higgins, eds., 1984); Animal Cell Culture (R. Freshney, ed., 1986); Perbal, A Practical Guide to Molecular Cloning (1984).

All publications, patents and patent applications cited herein, whether supra or infra, are hereby incorporated by reference in their entirety.

As used in this specification and the appended claims, the singular forms "a," "an" and "the" include plural references unless the content clearly dictates otherwise.

POLYPEPTIDE COMPOSITIONS

As used herein, the term "polypeptide" " is used in its conventional meaning, *i.e.*, as a sequence of amino acids. The polypeptides are not limited to a specific length of the product; thus, peptides, oligopeptides, and proteins are included within the definition of polypeptide, and such terms may be used interchangeably herein unless specifically indicated otherwise. This term also does not refer to or exclude post-expression modifications of the polypeptide, for example, glycosylations, acetylations, phosphorylations and the like, as well as other modifications known in the art, both naturally occurring and non-naturally occurring. A polypeptide may be an entire protein, or a subsequence thereof. Particular polypeptides of interest in the context of this invention are amino acid subsequences comprising epitopes, *i.e.*, antigenic determinants substantially responsible for the immunogenic properties of a polypeptide and being capable of evoking an immune response.

Particularly illustrative polypeptides of the present invention comprise those encoded by a polynucleotide sequence set forth in any one of SEQ ID NOs:1-234, 236, and 244, or a sequence that hybridizes under moderately stringent conditions, or, alternatively, under highly stringent conditions, to a polynucleotide sequence set forth in any one of SEQ ID NOs:1-234, 236, and 244. Certain other illustrative polypeptides of the invention comprise amino acid sequences as set forth in any one of SEQ ID NOs:235, 237, and 245.

The polypeptides of the present invention are sometimes herein referred to as colon tumor proteins or colon tumor polypeptides, as an indication that their identification has been based at least in part upon their increased levels of expression in colon tumor samples. Thus, a "colon tumor polypeptide" or "colon tumor protein," refers generally to a polypeptide sequence of the present invention, or a polynucleotide sequence encoding such a polypeptide, that is expressed in a substantial proportion of colon tumor samples, for example preferably greater than about 20%, more preferably greater than about 30%, and most preferably greater than about 50% or more of colon tumor samples tested, at a level that is at least two fold, and preferably at least five fold, greater than the level of expression in normal tissues, as determined using a representative assay provided herein. A colon tumor polypeptide sequence of the invention, based upon its increased level of expression in tumor cells, has particular utility both as a diagnostic marker as well as a therapeutic target, as further described below.

In certain preferred embodiments, the polypeptides of the invention are immunogenic, *i.e.*, they react detectably within an immunoassay (such as an ELISA or T-cell stimulation assay) with antisera and/or T-cells from a patient with colon cancer. Screening for immunogenic activity can be performed using techniques well known to the skilled artisan. For example, such screens can be performed using methods such as those described in Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988. In one illustrative example, a polypeptide may be immobilized on a solid support and contacted with patient sera to allow binding of antibodies within the sera to the immobilized polypeptide. Unbound sera may then be removed and bound antibodies detected using, for example, ^{125}I-labeled Protein A.

As would be recognized by the skilled artisan, immunogenic portions of the polypeptides disclosed herein are also encompassed by the present invention. An "immunogenic portion," as used herein, is a fragment of an immunogenic polypeptide of the invention that itself is immunologically reactive (*i.e.*, specifically binds) with the B-cells and/or T-cell surface antigen receptors that recognize the polypeptide. Immunogenic portions may generally be identified using well known techniques, such as those summarized in Paul, *Fundamental Immunology*, 3rd ed., 243-247 (Raven Press,

1993) and references cited therein. Such techniques include screening polypeptides for the ability to react with antigen-specific antibodies, antisera and/or T-cell lines or clones. As used herein, antisera and antibodies are "antigen-specific" if they specifically bind to an antigen (*i.e.*, they react with the protein in an ELISA or other immunoassay, and do not react detectably with unrelated proteins). Such antisera and antibodies may be prepared as described herein, and using well-known techniques.

In one preferred embodiment, an immunogenic portion of a polypeptide of the present invention is a portion that reacts with antisera and/or T-cells at a level that is not substantially less than the reactivity of the full-length polypeptide (*e.g.*, in an ELISA and/or T-cell reactivity assay). Preferably, the level of immunogenic activity of the immunogenic portion is at least about 50%, preferably at least about 70% and most preferably greater than about 90% of the immunogenicity for the full-length polypeptide. In some instances, preferred immunogenic portions will be identified that have a level of immunogenic activity greater than that of the corresponding full-length polypeptide, *e.g.*, having greater than about 100% or 150% or more immunogenic activity.

In certain other embodiments, illustrative immunogenic portions may include peptides in which an N-terminal leader sequence and/or transmembrane domain have been deleted. Other illustrative immunogenic portions will contain a small N- and/or C-terminal deletion (*e.g.*, 1-30 amino acids, preferably 5-15 amino acids), relative to the mature protein.

In another embodiment, a polypeptide composition of the invention may also comprise one or more polypeptides that are immunologically reactive with T cells and/or antibodies generated against a polypeptide of the invention, particularly a polypeptide having an amino acid sequence disclosed herein, or to an immunogenic fragment or variant thereof.

In another embodiment of the invention, polypeptides are provided that comprise one or more polypeptides that are capable of eliciting T cells and/or antibodies that are immunologically reactive with one or more polypeptides described herein, or one or more polypeptides encoded by contiguous nucleic acid sequences contained in the polynucleotide sequences disclosed herein, or immunogenic fragments

or variants thereof, or to one or more nucleic acid sequences which hybridize to one or more of these sequences under conditions of moderate to high stringency.

The present invention, in another aspect, provides polypeptide fragments comprising at least about 5, 10, 15, 20, 25, 50, or 100 contiguous amino acids, or more, including all intermediate lengths, of a polypeptide compositions set forth herein, such as those set forth in SEQ ID NOs:235, 237, and 245, or those encoded by a polynucleotide sequence set forth in a sequence of SEQ ID NOs:1-234, 236, and 244.

In another aspect, the present invention provides variants of the polypeptide compositions described herein. Polypeptide variants generally encompassed by the present invention will typically exhibit at least about 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% or more identity (determined as described below), along its length, to a polypeptide sequences set forth herein.

In one preferred embodiment, the polypeptide fragments and variants provided by the present invention are immunologically reactive with an antibody and/or T-cell that reacts with a full-length polypeptide specifically set forth herein.

In another preferred embodiment, the polypeptide fragments and variants provided by the present invention exhibit a level of immunogenic activity of at least about 50%, preferably at least about 70%, and most preferably at least about 90% or more of that exhibited by a full-length polypeptide sequence specifically set forth herein.

A polypeptide "variant," as the term is used herein, is a polypeptide that typically differs from a polypeptide specifically disclosed herein in one or more substitutions, deletions, additions and/or insertions. Such variants may be naturally occurring or may be synthetically generated, for example, by modifying one or more of the above polypeptide sequences of the invention and evaluating their immunogenic activity as described herein and/or using any of a number of techniques well known in the art.

For example, certain illustrative variants of the polypeptides of the invention include those in which one or more portions, such as an N-terminal leader sequence or transmembrane domain, have been removed. Other illustrative variants

include variants in which a small portion (*e.g.,* 1-30 amino acids, preferably 5-15 amino acids) has been removed from the N- and/or C-terminal of the mature protein.

In many instances, a variant will contain conservative substitutions. A "conservative substitution" is one in which an amino acid is substituted for another amino acid that has similar properties, such that one skilled in the art of peptide chemistry would expect the secondary structure and hydropathic nature of the polypeptide to be substantially unchanged. As described above, modifications may be made in the structure of the polynucleotides and polypeptides of the present invention and still obtain a functional molecule that encodes a variant or derivative polypeptide with desirable characteristics, *e.g.*, with immunogenic characteristics. When it is desired to alter the amino acid sequence of a polypeptide to create an equivalent, or even an improved, immunogenic variant or portion of a polypeptide of the invention, one skilled in the art will typically change one or more of the codons of the encoding DNA sequence according to Table 1.

For example, certain amino acids may be substituted for other amino acids in a protein structure without appreciable loss of interactive binding capacity with structures such as, for example, antigen-binding regions of antibodies or binding sites on substrate molecules. Since it is the interactive capacity and nature of a protein that defines that protein's biological functional activity, certain amino acid sequence substitutions can be made in a protein sequence, and, of course, its underlying DNA coding sequence, and nevertheless obtain a protein with like properties. It is thus contemplated that various changes may be made in the peptide sequences of the disclosed compositions, or corresponding DNA sequences which encode said peptides without appreciable loss of their biological utility or activity.

TABLE 1

Amino Acids			Codons					
Alanine	Ala	A	GCA	GCC	GCG	GCU		
Cysteine	Cys	C	UGC	UGU				
Aspartic acid	Asp	D	GAC	GAU				
Glutamic acid	Glu	E	GAA	GAG				
Phenylalanine	Phe	F	UUC	UUU				
Glycine	Gly	G	GGA	GGC	GGG	GGU		
Histidine	His	H	CAC	CAU				
Isoleucine	Ile	I	AUA	AUC	AUU			
Lysine	Lys	K	AAA	AAG				
Leucine	Leu	L	UUA	UUG	CUA	CUC	CUG	CUU
Methionine	Met	M	AUG					
Asparagine	Asn	N	AAC	AAU				
Proline	Pro	P	CCA	CCC	CCG	CCU		
Glutamine	Gln	Q	CAA	CAG				
Arginine	Arg	R	AGA	AGG	CGA	CGC	CGG	CGU
Serine	Ser	S	AGC	AGU	UCA	UCC	UCG	UCU
Threonine	Thr	T	ACA	ACC	ACG	ACU		
Valine	Val	V	GUA	GUC	GUG	GUU		
Tryptophan	Trp	W	UGG					
Tyrosine	Tyr	Y	UAC	UAU				

In making such changes, the hydropathic index of amino acids may be considered. The importance of the hydropathic amino acid index in conferring interactive biologic function on a protein is generally understood in the art (Kyte and Doolittle, 1982, incorporated herein by reference). It is accepted that the relative hydropathic character of the amino acid contributes to the secondary structure of the resultant protein, which in turn defines the interaction of the protein with other molecules, for example, enzymes, substrates, receptors, DNA, antibodies, antigens, and the like. Each amino acid has been assigned a hydropathic index on the basis of its hydrophobicity and charge characteristics (Kyte and Doolittle, 1982). These values are:

isoleucine (+4.5); valine (+4.2); leucine (+3.8); phenylalanine (+2.8); cysteine/cystine (+2.5); methionine (+1.9); alanine (+1.8); glycine (–0.4); threonine (–0.7); serine (–0.8); tryptophan (–0.9); tyrosine (–1.3); proline (–1.6); histidine (–3.2); glutamate (–3.5); glutamine (–3.5); aspartate (–3.5); asparagine (–3.5); lysine (–3.9); and arginine (–4.5).

It is known in the art that certain amino acids may be substituted by other amino acids having a similar hydropathic index or score and still result in a protein with similar biological activity, *i.e.* still obtain a biological functionally equivalent protein. In making such changes, the substitution of amino acids whose hydropathic indices are within ±2 is preferred, those within ±1 are particularly preferred, and those within ±0.5 are even more particularly preferred. It is also understood in the art that the substitution of like amino acids can be made effectively on the basis of hydrophilicity. U. S. Patent 4,554,101 (specifically incorporated herein by reference in its entirety), states that the greatest local average hydrophilicity of a protein, as governed by the hydrophilicity of its adjacent amino acids, correlates with a biological property of the protein.

As detailed in U. S. Patent 4,554,101, the following hydrophilicity values have been assigned to amino acid residues: arginine (+3.0); lysine (+3.0); aspartate (+3.0 ± 1); glutamate (+3.0 ± 1); serine (+0.3); asparagine (+0.2); glutamine (+0.2); glycine (0); threonine (–0.4); proline (–0.5 ± 1); alanine (–0.5); histidine (–0.5); cysteine (–1.0); methionine (–1.3); valine (–1.5); leucine (–1.8); isoleucine (–1.8); tyrosine (–2.3); phenylalanine (–2.5); tryptophan (–3.4). It is understood that an amino acid can be substituted for another having a similar hydrophilicity value and still obtain a biologically equivalent, and in particular, an immunologically equivalent protein. In such changes, the substitution of amino acids whose hydrophilicity values are within ±2 is preferred, those within ±1 are particularly preferred, and those within ±0.5 are even more particularly preferred.

As outlined above, amino acid substitutions are generally therefore based on the relative similarity of the amino acid side-chain substituents, for example, their hydrophobicity, hydrophilicity, charge, size, and the like. Exemplary substitutions that take various of the foregoing characteristics into consideration are well known to those

of skill in the art and include: arginine and lysine; glutamate and aspartate; serine and threonine; glutamine and asparagine; and valine, leucine and isoleucine.

In addition, any polynucleotide may be further modified to increase stability *in vivo*. Possible modifications include, but are not limited to, the addition of flanking sequences at the 5' and/or 3' ends; the use of phosphorothioate or 2' O-methyl rather than phosphodiesterase linkages in the backbone; and/or the inclusion of nontraditional bases such as inosine, queosine and wybutosine, as well as acetyl-methyl-, thio- and other modified forms of adenine, cytidine, guanine, thymine and uridine.

Amino acid substitutions may further be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity and/or the amphipathic nature of the residues. For example, negatively charged amino acids include aspartic acid and glutamic acid; positively charged amino acids include lysine and arginine; and amino acids with uncharged polar head groups having similar hydrophilicity values include leucine, isoleucine and valine; glycine and alanine; asparagine and glutamine; and serine, threonine, phenylalanine and tyrosine. Other groups of amino acids that may represent conservative changes include: (1) ala, pro, gly, glu, asp, gln, asn, ser, thr; (2) cys, ser, tyr, thr; (3) val, ile, leu, met, ala, phe; (4) lys, arg, his; and (5) phe, tyr, trp, his. A variant may also, or alternatively, contain nonconservative changes. In a preferred embodiment, variant polypeptides differ from a native sequence by substitution, deletion or addition of five amino acids or fewer. Variants may also (or alternatively) be modified by, for example, the deletion or addition of amino acids that have minimal influence on the immunogenicity, secondary structure and hydropathic nature of the polypeptide.

As noted above, polypeptides may comprise a signal (or leader) sequence at the N-terminal end of the protein, which co-translationally or post-translationally directs transfer of the protein. The polypeptide may also be conjugated to a linker or other sequence for ease of synthesis, purification or identification of the polypeptide (*e.g.*, poly-His), or to enhance binding of the polypeptide to a solid support. For example, a polypeptide may be conjugated to an immunoglobulin Fc region.

When comparing polypeptide sequences, two sequences are said to be "identical" if the sequence of amino acids in the two sequences is the same when aligned for maximum correspondence, as described below. Comparisons between two sequences are typically performed by comparing the sequences over a comparison window to identify and compare local regions of sequence similarity. A "comparison window" as used herein, refers to a segment of at least about 20 contiguous positions, usually 30 to about 75, 40 to about 50, in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned.

Optimal alignment of sequences for comparison may be conducted using the Megalign program in the Lasergene suite of bioinformatics software (DNASTAR, Inc., Madison, WI), using default parameters. This program embodies several alignment schemes described in the following references: Dayhoff, M.O. (1978) A model of evolutionary change in proteins – Matrices for detecting distant relationships. In Dayhoff, M.O. (ed.) Atlas of Protein Sequence and Structure, National Biomedical Research Foundation, Washington DC Vol. 5, Suppl. 3, pp. 345-358; Hein J. (1990) Unified Approach to Alignment and Phylogenes pp. 626-645 *Methods in Enzymology* vol. 183, Academic Press, Inc., San Diego, CA; Higgins, D.G. and Sharp, P.M. (1989) *CABIOS 5*:151-153; Myers, E.W. and Muller W. (1988) *CABIOS 4*:11-17; Robinson, E.D. (1971) *Comb. Theor 11*:105; Saitou, N. Nei, M. (1987) *Mol. Biol. Evol. 4*:406-425; Sneath, P.H.A. and Sokal, R.R. (1973) *Numerical Taxonomy – the Principles and Practice of Numerical Taxonomy*, Freeman Press, San Francisco, CA; Wilbur, W.J. and Lipman, D.J. (1983) *Proc. Natl. Acad., Sci. USA 80*:726-730.

Alternatively, optimal alignment of sequences for comparison may be conducted by the local identity algorithm of Smith and Waterman (1981) *Add. APL. Math* 2:482, by the identity alignment algorithm of Needleman and Wunsch (1970) *J. Mol. Biol.* 48:443, by the search for similarity methods of Pearson and Lipman (1988) *Proc. Natl. Acad. Sci. USA* 85: 2444, by computerized implementations of these algorithms (GAP, BESTFIT, BLAST, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group (GCG), 575 Science Dr., Madison, WI), or by inspection.

One preferred example of algorithms that are suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul et al. (1977) *Nucl. Acids Res.* 25:3389-3402 and Altschul et al. (1990) *J. Mol. Biol.* 215:403-410, respectively. BLAST and BLAST 2.0 can be used, for example with the parameters described herein, to determine percent sequence identity for the polynucleotides and polypeptides of the invention. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information. For amino acid sequences, a scoring matrix can be used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T and X determine the sensitivity and speed of the alignment.

In one preferred approach, the "percentage of sequence identity" is determined by comparing two optimally aligned sequences over a window of comparison of at least 20 positions, wherein the portion of the polypeptide sequence in the comparison window may comprise additions or deletions (*i.e.*, gaps) of 20 percent or less, usually 5 to 15 percent, or 10 to 12 percent, as compared to the reference sequences (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the reference sequence (*i.e.*, the window size) and multiplying the results by 100 to yield the percentage of sequence identity.

Within other illustrative embodiments, a polypeptide may be a xenogeneic polypeptide that comprises an polypeptide having substantial sequence identity, as described above, to the human polypeptide (also termed autologous antigen) which served as a reference polypeptide, but which xenogeneic polypeptide is derived from a different, non-human species. One skilled in the art will recognize that "self"antigens are often poor stimulators of CD8+ and CD4+ T-lymphocyte responses,

and therefore efficient immunotherapeutic strategies directed against tumor polypeptides require the development of methods to overcome immune tolerance to particular self tumor polypeptides. For example, humans immunized with prostase protein from a xenogeneic (non human) origin are capable of mounting an immune response against the counterpart human protein, *e.g.* the human prostase tumor protein present on human tumor cells. Accordingly, the present invention provides methods for purifying the xenogeneic form of the tumor proteins set forth herein, such as the polypeptides set forth in SEQ ID NOs:235, 237, and 245, or those encoded by polynucleotide sequences set forth in SEQ ID NOs:1-234, 236, and 244.

Therefore, one aspect of the present invention provides xenogeneic variants of the polypeptide compositions described herein. Such xenogeneic variants generally encompassed by the present invention will typically exhibit at least about 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% or more identity along their lengths, to a polypeptide sequences set forth herein.

More particularly, the invention is directed to mouse, rat, monkey, porcine and other non-human polypeptides which can be used as xenogeneic forms of human polypeptides set forth herein, to induce immune responses directed against tumor polypeptides of the invention.

Within other illustrative embodiments, a polypeptide may be a fusion polypeptide that comprises multiple polypeptides as described herein, or that comprises at least one polypeptide as described herein and an unrelated sequence, such as a known tumor protein. A fusion partner may, for example, assist in providing T helper epitopes (an immunological fusion partner), preferably T helper epitopes recognized by humans, or may assist in expressing the protein (an expression enhancer) at higher yields than the native recombinant protein. Certain preferred fusion partners are both immunological and expression enhancing fusion partners. Other fusion partners may be selected so as to increase the solubility of the polypeptide or to enable the polypeptide to be targeted to desired intracellular compartments. Still further fusion partners include affinity tags, which facilitate purification of the polypeptide.

Fusion polypeptides may generally be prepared using standard techniques, including chemical conjugation. Preferably, a fusion polypeptide is

expressed as a recombinant polypeptide, allowing the production of increased levels, relative to a non-fused polypeptide, in an expression system. Briefly, DNA sequences encoding the polypeptide components may be assembled separately, and ligated into an appropriate expression vector. The 3' end of the DNA sequence encoding one polypeptide component is ligated, with or without a peptide linker, to the 5' end of a DNA sequence encoding the second polypeptide component so that the reading frames of the sequences are in phase. This permits translation into a single fusion polypeptide that retains the biological activity of both component polypeptides.

A peptide linker sequence may be employed to separate the first and second polypeptide components by a distance sufficient to ensure that each polypeptide folds into its secondary and tertiary structures. Such a peptide linker sequence is incorporated into the fusion polypeptide using standard techniques well known in the art. Suitable peptide linker sequences may be chosen based on the following factors: (1) their ability to adopt a flexible extended conformation; (2) their inability to adopt a secondary structure that could interact with functional epitopes on the first and second polypeptides; and (3) the lack of hydrophobic or charged residues that might react with the polypeptide functional epitopes. Preferred peptide linker sequences contain Gly, Asn and Ser residues. Other near neutral amino acids, such as Thr and Ala may also be used in the linker sequence. Amino acid sequences which may be usefully employed as linkers include those disclosed in Maratea et al., *Gene 40*:39-46, 1985; Murphy et al., *Proc. Natl. Acad. Sci. USA 83*:8258-8262, 1986; U.S. Patent No. 4,935,233 and U.S. Patent No. 4,751,180. The linker sequence may generally be from 1 to about 50 amino acids in length. Linker sequences are not required when the first and second polypeptides have non-essential N-terminal amino acid regions that can be used to separate the functional domains and prevent steric interference.

The ligated DNA sequences are operably linked to suitable transcriptional or translational regulatory elements. The regulatory elements responsible for expression of DNA are located only 5' to the DNA sequence encoding the first polypeptides. Similarly, stop codons required to end translation and transcription termination signals are only present 3' to the DNA sequence encoding the second polypeptide.

The fusion polypeptide can comprise a polypeptide as described herein together with an unrelated immunogenic protein, such as an immunogenic protein capable of eliciting a recall response. Examples of such proteins include tetanus, tuberculosis and hepatitis proteins (*see*, for example, Stoute et al. *New Engl. J. Med.*, *336*:86-91, 1997).

In one preferred embodiment, the immunological fusion partner is derived from a Mycobacterium sp., such as a Mycobacterium tuberculosis-derived Ra12 fragment. Ra12 compositions and methods for their use in enhancing the expression and/or immunogenicity of heterologous polynucleotide/polypeptide sequences is described in U.S. Patent Application 60/158,585, the disclosure of which is incorporated herein by reference in its entirety. Briefly, Ra12 refers to a polynucleotide region that is a subsequence of a *Mycobacterium tuberculosis* MTB32A nucleic acid. MTB32A is a serine protease of 32 KD molecular weight encoded by a gene in virulent and avirulent strains of *M. tuberculosis*. The nucleotide sequence and amino acid sequence of MTB32A have been described (for example, U.S. Patent Application 60/158,585; see also, Skeiky *et al.*, *Infection and Immun.* (1999) 67:3998-4007, incorporated herein by reference). C-terminal fragments of the MTB32A coding sequence express at high levels and remain as a soluble polypeptides throughout the purification process. Moreover, Ra12 may enhance the immunogenicity of heterologous immunogenic polypeptides with which it is fused. One preferred Ra12 fusion polypeptide comprises a 14 KD C-terminal fragment corresponding to amino acid residues 192 to 323 of MTB32A. Other preferred Ra12 polynucleotides generally comprise at least about 15 consecutive nucleotides, at least about 30 nucleotides, at least about 60 nucleotides, at least about 100 nucleotides, at least about 200 nucleotides, or at least about 300 nucleotides that encode a portion of a Ra12 polypeptide. Ra12 polynucleotides may comprise a native sequence (*i.e.*, an endogenous sequence that encodes a Ra12 polypeptide or a portion thereof) or may comprise a variant of such a sequence. Ra12 polynucleotide variants may contain one or more substitutions, additions, deletions and/or insertions such that the biological activity of the encoded fusion polypeptide is not substantially diminished, relative to a fusion polypeptide comprising a native Ra12 polypeptide. Variants preferably exhibit at least about 70%

identity, more preferably at least about 80% identity and most preferably at least about 90% identity to a polynucleotide sequence that encodes a native Ra12 polypeptide or a portion thereof.

Within other preferred embodiments, an immunological fusion partner is derived from protein D, a surface protein of the gram-negative bacterium Haemophilus influenza B (WO 91/18926). Preferably, a protein D derivative comprises approximately the first third of the protein (*e.g.,* the first N-terminal 100-110 amino acids), and a protein D derivative may be lipidated. Within certain preferred embodiments, the first 109 residues of a Lipoprotein D fusion partner is included on the N-terminus to provide the polypeptide with additional exogenous T-cell epitopes and to increase the expression level in *E. coli* (thus functioning as an expression enhancer). The lipid tail ensures optimal presentation of the antigen to antigen presenting cells. Other fusion partners include the non-structural protein from influenzae virus, NS1 (hemaglutinin). Typically, the N-terminal 81 amino acids are used, although different fragments that include T-helper epitopes may be used.

In another embodiment, the immunological fusion partner is the protein known as LYTA, or a portion thereof (preferably a C-terminal portion). LYTA is derived from *Streptococcus pneumoniae,* which synthesizes an N-acetyl-L-alanine amidase known as amidase LYTA (encoded by the LytA gene; *Gene 43*:265-292, 1986). LYTA is an autolysin that specifically degrades certain bonds in the peptidoglycan backbone. The C-terminal domain of the LYTA protein is responsible for the affinity to the choline or to some choline analogues such as DEAE. This property has been exploited for the development of *E. coli* C-LYTA expressing plasmids useful for expression of fusion proteins. Purification of hybrid proteins containing the C-LYTA fragment at the amino terminus has been described (*see Biotechnology 10*:795-798, 1992). Within a preferred embodiment, a repeat portion of LYTA may be incorporated into a fusion polypeptide. A repeat portion is found in the C-terminal region starting at residue 178. A particularly preferred repeat portion incorporates residues 188-305.

Yet another illustrative embodiment involves fusion polypeptides, and the polynucleotides encoding them, wherein the fusion partner comprises a targeting

signal capable of directing a polypeptide to the endosomal/lysosomal compartment, as described in U.S. Patent No. 5,633,234. An immunogenic polypeptide of the invention, when fused with this targeting signal, will associate more efficiently with MHC class II molecules and thereby provide enhanced in vivo stimulation of $CD4^+$ T-cells specific for the polypeptide.

Polypeptides of the invention are prepared using any of a variety of well known synthetic and/or recombinant techniques, the latter of which are further described below. Polypeptides, portions and other variants generally less than about 150 amino acids can be generated by synthetic means, using techniques well known to those of ordinary skill in the art. In one illustrative example, such polypeptides are synthesized using any of the commercially available solid-phase techniques, such as the Merrifield solid-phase synthesis method, where amino acids are sequentially added to a growing amino acid chain. *See* Merrifield, *J. Am. Chem. Soc. 85*:2149-2146, 1963. Equipment for automated synthesis of polypeptides is commercially available from suppliers such as Perkin Elmer/Applied BioSystems Division (Foster City, CA), and may be operated according to the manufacturer's instructions.

In general, polypeptide compositions (including fusion polypeptides) of the invention are isolated. An "isolated" polypeptide is one that is removed from its original environment. For example, a naturally-occurring protein or polypeptide is isolated if it is separated from some or all of the coexisting materials in the natural system. Preferably, such polypeptides are also purified, *e.g.*, are at least about 90% pure, more preferably at least about 95% pure and most preferably at least about 99% pure.

POLYNUCLEOTIDE COMPOSITIONS

The present invention, in other aspects, provides polynucleotide compositions. The terms "DNA" and "polynucleotide" are used essentially interchangeably herein to refer to a DNA molecule that has been isolated free of total genomic DNA of a particular species. "Isolated," as used herein, means that a polynucleotide is substantially away from other coding sequences, and that the DNA molecule does not contain large portions of unrelated coding DNA, such as large

chromosomal fragments or other functional genes or polypeptide coding regions. Of course, this refers to the DNA molecule as originally isolated, and does not exclude genes or coding regions later added to the segment by the hand of man.

As will be understood by those skilled in the art, the polynucleotide compositions of this invention can include genomic sequences, extra-genomic and plasmid-encoded sequences and smaller engineered gene segments that express, or may be adapted to express, proteins, polypeptides, peptides and the like. Such segments may be naturally isolated, or modified synthetically by the hand of man.

As will be also recognized by the skilled artisan, polynucleotides of the invention may be single-stranded (coding or antisense) or double-stranded, and may be DNA (genomic, cDNA or synthetic) or RNA molecules. RNA molecules may include HnRNA molecules, which contain introns and correspond to a DNA molecule in a one-to-one manner, and mRNA molecules, which do not contain introns. Additional coding or non-coding sequences may, but need not, be present within a polynucleotide of the present invention, and a polynucleotide may, but need not, be linked to other molecules and/or support materials.

Polynucleotides may comprise a native sequence (*i.e.*, an endogenous sequence that encodes a polypeptide/protein of the invention or a portion thereof) or may comprise a sequence that encodes a variant or derivative, preferably and immunogenic variant or derivative, of such a sequence.

Therefore, according to another aspect of the present invention, polynucleotide compositions are provided that comprise some or all of a polynucleotide sequence set forth in any one of SEQ ID NOs:1-234, 236, and 244, complements of a polynucleotide sequence set forth in any one of SEQ ID NOs:1-234, 236, and 244, and degenerate variants of a polynucleotide sequence set forth in any one of SEQ ID NOs:1-234, 236, and 244. In certain preferred embodiments, the polynucleotide sequences set forth herein encode immunogenic polypeptides, as described above.

In other related embodiments, the present invention provides polynucleotide variants having substantial identity to the sequences disclosed herein in SEQ ID NOs:1-234, 236, and 244, for example those comprising at least 70% sequence identity, preferably at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% or

higher, sequence identity compared to a polynucleotide sequence of this invention using the methods described herein, (*e.g.*, BLAST analysis using standard parameters, as described below). One skilled in this art will recognize that these values can be appropriately adjusted to determine corresponding identity of proteins encoded by two nucleotide sequences by taking into account codon degeneracy, amino acid similarity, reading frame positioning and the like.

Typically, polynucleotide variants will contain one or more substitutions, additions, deletions and/or insertions, preferably such that the immunogenicity of the polypeptide encoded by the variant polynucleotide is not substantially diminished relative to a polypeptide encoded by a polynucleotide sequence specifically set forth herein). The term "variants" should also be understood to encompasses homologous genes of xenogeneic origin.

In additional embodiments, the present invention provides polynucleotide fragments comprising or consisting of various lengths of contiguous stretches of sequence identical to or complementary to one or more of the sequences disclosed herein. For example, polynucleotides are provided by this invention that comprise or consist of at least about 10, 15, 20, 30, 40, 50, 75, 100, 150, 200, 300, 400, 500 or 1000 or more contiguous nucleotides of one or more of the sequences disclosed herein as well as all intermediate lengths there between. It will be readily understood that "intermediate lengths", in this context, means any length between the quoted values, such as 16, 17, 18, 19, *etc.*; 21, 22, 23, *etc.*; 30, 31, 32, *etc.*; 50, 51, 52, 53, *etc.*; 100, 101, 102, 103, *etc.*; 150, 151, 152, 153, *etc.*; including all integers through 200-500; 500-1,000, and the like. A polynucleotide sequence as described here may be extended at one or both ends by additional nucleotides not found in the native sequence. This additional sequence may consist of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 nucleotides at either end of the disclosed sequence or at both ends of the disclosed sequence.

In another embodiment of the invention, polynucleotide compositions are provided that are capable of hybridizing under moderate to high stringency conditions to a polynucleotide sequence provided herein, or a fragment thereof, or a complementary sequence thereof. Hybridization techniques are well known in the art

of molecular biology. For purposes of illustration, suitable moderately stringent conditions for testing the hybridization of a polynucleotide of this invention with other polynucleotides include prewashing in a solution of 5 X SSC, 0.5% SDS, 1.0 mM EDTA (pH 8.0); hybridizing at 50°C-60°C, 5 X SSC, overnight; followed by washing twice at 65°C for 20 minutes with each of 2X, 0.5X and 0.2X SSC containing 0.1% SDS. One skilled in the art will understand that the stringency of hybridization can be readily manipulated, such as by altering the salt content of the hybridization solution and/or the temperature at which the hybridization is performed. For example, in another embodiment, suitable highly stringent hybridization conditions include those described above, with the exception that the temperature of hybridization is increased, *e.g.*, to 60-65°C or 65-70°C.

In certain preferred embodiments, the polynucleotides described above, *e.g.*, polynucleotide variants, fragments and hybridizing sequences, encode polypeptides that are immunologically cross-reactive with a polypeptide sequence specifically set forth herein. In other preferred embodiments, such polynucleotides encode polypeptides that have a level of immunogenic activity of at least about 50%, preferably at least about 70%, and more preferably at least about 90% of that for a polypeptide sequence specifically set forth herein.

The polynucleotides of the present invention, or fragments thereof, regardless of the length of the coding sequence itself, may be combined with other DNA sequences, such as promoters, polyadenylation signals, additional restriction enzyme sites, multiple cloning sites, other coding segments, and the like, such that their overall length may vary considerably. It is therefore contemplated that a nucleic acid fragment of almost any length may be employed, with the total length preferably being limited by the ease of preparation and use in the intended recombinant DNA protocol. For example, illustrative polynucleotide segments with total lengths of about 10,000, about 5000, about 3000, about 2,000, about 1,000, about 500, about 200, about 100, about 50 base pairs in length, and the like, (including all intermediate lengths) are contemplated to be useful in many implementations of this invention.

When comparing polynucleotide sequences, two sequences are said to be "identical" if the sequence of nucleotides in the two sequences is the same when aligned

for maximum correspondence, as described below. Comparisons between two sequences are typically performed by comparing the sequences over a comparison window to identify and compare local regions of sequence similarity. A "comparison window" as used herein, refers to a segment of at least about 20 contiguous positions, usually 30 to about 75, 40 to about 50, in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned.

Optimal alignment of sequences for comparison may be conducted using the Megalign program in the Lasergene suite of bioinformatics software (DNASTAR, Inc., Madison, WI), using default parameters. This program embodies several alignment schemes described in the following references: Dayhoff, M.O. (1978) A model of evolutionary change in proteins – Matrices for detecting distant relationships. In Dayhoff, M.O. (ed.) Atlas of Protein Sequence and Structure, National Biomedical Research Foundation, Washington DC Vol. 5, Suppl. 3, pp. 345-358; Hein J. (1990) Unified Approach to Alignment and Phylogenes pp. 626-645 *Methods in Enzymology* vol. 183, Academic Press, Inc., San Diego, CA; Higgins, D.G. and Sharp, P.M. (1989) *CABIOS 5*:151-153; Myers, E.W. and Muller W. (1988) *CABIOS 4*:11-17; Robinson, E.D. (1971) *Comb. Theor 11*:105; Santou, N. Nes, M. (1987) *Mol. Biol. Evol. 4*:406-425; Sneath, P.H.A. and Sokal, R.R. (1973) *Numerical Taxonomy – the Principles and Practice of Numerical Taxonomy*, Freeman Press, San Francisco, CA; Wilbur, W.J. and Lipman, D.J. (1983) *Proc. Natl. Acad., Sci. USA 80*:726-730.

Alternatively, optimal alignment of sequences for comparison may be conducted by the local identity algorithm of Smith and Waterman (1981) *Add. APL. Math* 2:482, by the identity alignment algorithm of Needleman and Wunsch (1970) *J. Mol. Biol.* 48:443, by the search for similarity methods of Pearson and Lipman (1988) *Proc. Natl. Acad. Sci. USA* 85: 2444, by computerized implementations of these algorithms (GAP, BESTFIT, BLAST, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group (GCG), 575 Science Dr., Madison, WI), or by inspection.

One preferred example of algorithms that are suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0

algorithms, which are described in Altschul et al. (1977) *Nucl. Acids Res.* 25:3389-3402 and Altschul et al. (1990) *J. Mol. Biol.* 215:403-410, respectively. BLAST and BLAST 2.0 can be used, for example with the parameters described herein, to determine percent sequence identity for the polynucleotides of the invention. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information. In one illustrative example, cumulative scores can be calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always >0) and N (penalty score for mismatching residues; always <0). Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, and expectation (E) of 10, and the BLOSUM62 scoring matrix (see Henikoff and Henikoff (1989) *Proc. Natl. Acad. Sci. USA* 89:10915) alignments, (B) of 50, expectation (E) of 10, M=5, N=-4 and a comparison of both strands.

Preferably, the "percentage of sequence identity" is determined by comparing two optimally aligned sequences over a window of comparison of at least 20 positions, wherein the portion of the polynucleotide sequence in the comparison window may comprise additions or deletions (*i.e.*, gaps) of 20 percent or less, usually 5 to 15 percent, or 10 to 12 percent, as compared to the reference sequences (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid bases occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the reference sequence (*i.e.*, the window size) and multiplying the results by 100 to yield the percentage of sequence identity.

It will be appreciated by those of ordinary skill in the art that, as a result of the degeneracy of the genetic code, there are many nucleotide sequences that encode a polypeptide as described herein. Some of these polynucleotides bear minimal

homology to the nucleotide sequence of any native gene. Nonetheless, polynucleotides that vary due to differences in codon usage are specifically contemplated by the present invention. Further, alleles of the genes comprising the polynucleotide sequences provided herein are within the scope of the present invention. Alleles are endogenous genes that are altered as a result of one or more mutations, such as deletions, additions and/or substitutions of nucleotides. The resulting mRNA and protein may, but need not, have an altered structure or function. Alleles may be identified using standard techniques (such as hybridization, amplification and/or database sequence comparison).

Therefore, in another embodiment of the invention, a mutagenesis approach, such as site-specific mutagenesis, is employed for the preparation of immunogenic variants and/or derivatives of the polypeptides described herein. By this approach, specific modifications in a polypeptide sequence can be made through mutagenesis of the underlying polynucleotides that encode them. These techniques provides a straightforward approach to prepare and test sequence variants, for example, incorporating one or more of the foregoing considerations, by introducing one or more nucleotide sequence changes into the polynucleotide.

Site-specific mutagenesis allows the production of mutants through the use of specific oligonucleotide sequences which encode the DNA sequence of the desired mutation, as well as a sufficient number of adjacent nucleotides, to provide a primer sequence of sufficient size and sequence complexity to form a stable duplex on both sides of the deletion junction being traversed. Mutations may be employed in a selected polynucleotide sequence to improve, alter, decrease, modify, or otherwise change the properties of the polynucleotide itself, and/or alter the properties, activity, composition, stability, or primary sequence of the encoded polypeptide.

In certain embodiments of the present invention, the inventors contemplate the mutagenesis of the disclosed polynucleotide sequences to alter one or more properties of the encoded polypeptide, such as the immunogenicity of a polypeptide vaccine. The techniques of site-specific mutagenesis are well-known in the art, and are widely used to create variants of both polypeptides and polynucleotides. For example, site-specific mutagenesis is often used to alter a specific portion of a DNA molecule. In such embodiments, a primer comprising typically about 14 to about 25

nucleotides or so in length is employed, with about 5 to about 10 residues on both sides of the junction of the sequence being altered.

As will be appreciated by those of skill in the art, site-specific mutagenesis techniques have often employed a phage vector that exists in both a single stranded and double stranded form. Typical vectors useful in site-directed mutagenesis include vectors such as the M13 phage. These phage are readily commercially-available and their use is generally well-known to those skilled in the art. Double-stranded plasmids are also routinely employed in site directed mutagenesis that eliminates the step of transferring the gene of interest from a plasmid to a phage.

In general, site-directed mutagenesis in accordance herewith is performed by first obtaining a single-stranded vector or melting apart of two strands of a double-stranded vector that includes within its sequence a DNA sequence that encodes the desired peptide. An oligonucleotide primer bearing the desired mutated sequence is prepared, generally synthetically. This primer is then annealed with the single-stranded vector, and subjected to DNA polymerizing enzymes such as *E. coli* polymerase I Klenow fragment, in order to complete the synthesis of the mutation-bearing strand. Thus, a heteroduplex is formed wherein one strand encodes the original non-mutated sequence and the second strand bears the desired mutation. This heteroduplex vector is then used to transform appropriate cells, such as *E. coli* cells, and clones are selected which include recombinant vectors bearing the mutated sequence arrangement.

The preparation of sequence variants of the selected peptide-encoding DNA segments using site-directed mutagenesis provides a means of producing potentially useful species and is not meant to be limiting as there are other ways in which sequence variants of peptides and the DNA sequences encoding them may be obtained. For example, recombinant vectors encoding the desired peptide sequence may be treated with mutagenic agents, such as hydroxylamine, to obtain sequence variants. Specific details regarding these methods and protocols are found in the teachings of Maloy *et al.*, 1994; Segal, 1976; Prokop and Bajpai, 1991; Kuby, 1994; and Maniatis *et al.*, 1982, each incorporated herein by reference, for that purpose.

As used herein, the term "oligonucleotide directed mutagenesis procedure" refers to template-dependent processes and vector-mediated propagation which result in an increase in the concentration of a specific nucleic acid molecule relative to its initial concentration, or in an increase in the concentration of a detectable signal, such as amplification. As used herein, the term "oligonucleotide directed mutagenesis procedure" is intended to refer to a process that involves the template-dependent extension of a primer molecule. The term template dependent process refers to nucleic acid synthesis of an RNA or a DNA molecule wherein the sequence of the newly synthesized strand of nucleic acid is dictated by the well-known rules of complementary base pairing (see, for example, Watson, 1987). Typically, vector mediated methodologies involve the introduction of the nucleic acid fragment into a DNA or RNA vector, the clonal amplification of the vector, and the recovery of the amplified nucleic acid fragment. Examples of such methodologies are provided by U. S. Patent No. 4,237,224, specifically incorporated herein by reference in its entirety.

In another approach for the production of polypeptide variants of the present invention, recursive sequence recombination, as described in U.S. Patent No. 5,837,458, may be employed. In this approach, iterative cycles of recombination and screening or selection are performed to "evolve" individual polynucleotide variants of the invention having, for example, enhanced immunogenic activity.

In other embodiments of the present invention, the polynucleotide sequences provided herein can be advantageously used as probes or primers for nucleic acid hybridization. As such, it is contemplated that nucleic acid segments that comprise or consist of a sequence region of at least about a 15 nucleotide long contiguous sequence that has the same sequence as, or is complementary to, a 15 nucleotide long contiguous sequence disclosed herein will find particular utility. Longer contiguous identical or complementary sequences, *e.g.*, those of about 20, 30, 40, 50, 100, 200, 500, 1000 (including all intermediate lengths) and even up to full length sequences will also be of use in certain embodiments.

The ability of such nucleic acid probes to specifically hybridize to a sequence of interest will enable them to be of use in detecting the presence of complementary sequences in a given sample. However, other uses are also envisioned,

such as the use of the sequence information for the preparation of mutant species primers, or primers for use in preparing other genetic constructions.

Polynucleotide molecules having sequence regions consisting of contiguous nucleotide stretches of 10-14, 15-20, 30, 50, or even of 100-200 nucleotides or so (including intermediate lengths as well), identical or complementary to a polynucleotide sequence disclosed herein, are particularly contemplated as hybridization probes for use in, *e.g.*, Southern and Northern blotting. This would allow a gene product, or fragment thereof, to be analyzed, both in diverse cell types and also in various bacterial cells. The total size of fragment, as well as the size of the complementary stretch(es), will ultimately depend on the intended use or application of the particular nucleic acid segment. Smaller fragments will generally find use in hybridization embodiments, wherein the length of the contiguous complementary region may be varied, such as between about 15 and about 100 nucleotides, but larger contiguous complementarity stretches may be used, according to the length complementary sequences one wishes to detect.

The use of a hybridization probe of about 15-25 nucleotides in length allows the formation of a duplex molecule that is both stable and selective. Molecules having contiguous complementary sequences over stretches greater than 15 bases in length are generally preferred, though, in order to increase stability and selectivity of the hybrid, and thereby improve the quality and degree of specific hybrid molecules obtained. One will generally prefer to design nucleic acid molecules having gene-complementary stretches of 15 to 25 contiguous nucleotides, or even longer where desired.

Hybridization probes may be selected from any portion of any of the sequences disclosed herein. All that is required is to review the sequences set forth herein, or to any continuous portion of the sequences, from about 15-25 nucleotides in length up to and including the full length sequence, that one wishes to utilize as a probe or primer. The choice of probe and primer sequences may be governed by various factors. For example, one may wish to employ primers from towards the termini of the total sequence.

Small polynucleotide segments or fragments may be readily prepared by, for example, directly synthesizing the fragment by chemical means, as is commonly practiced using an automated oligonucleotide synthesizer. Also, fragments may be obtained by application of nucleic acid reproduction technology, such as the PCR™ technology of U. S. Patent 4,683,202 (incorporated herein by reference), by introducing selected sequences into recombinant vectors for recombinant production, and by other recombinant DNA techniques generally known to those of skill in the art of molecular biology.

The nucleotide sequences of the invention may be used for their ability to selectively form duplex molecules with complementary stretches of the entire gene or gene fragments of interest. Depending on the application envisioned, one will typically desire to employ varying conditions of hybridization to achieve varying degrees of selectivity of probe towards target sequence. For applications requiring high selectivity, one will typically desire to employ relatively stringent conditions to form the hybrids, *e.g.*, one will select relatively low salt and/or high temperature conditions, such as provided by a salt concentration of from about 0.02 M to about 0.15 M salt at temperatures of from about 50°C to about 70°C. Such selective conditions tolerate little, if any, mismatch between the probe and the template or target strand, and would be particularly suitable for isolating related sequences.

Of course, for some applications, for example, where one desires to prepare mutants employing a mutant primer strand hybridized to an underlying template, less stringent (reduced stringency) hybridization conditions will typically be needed in order to allow formation of the heteroduplex. In these circumstances, one may desire to employ salt conditions such as those of from about 0.15 M to about 0.9 M salt, at temperatures ranging from about 20°C to about 55°C. Cross-hybridizing species can thereby be readily identified as positively hybridizing signals with respect to control hybridizations. In any case, it is generally appreciated that conditions can be rendered more stringent by the addition of increasing amounts of formamide, which serves to destabilize the hybrid duplex in the same manner as increased temperature. Thus, hybridization conditions can be readily manipulated, and thus will generally be a method of choice depending on the desired results.

According to another embodiment of the present invention, polynucleotide compositions comprising antisense oligonucleotides are provided. Antisense oligonucleotides have been demonstrated to be effective and targeted inhibitors of protein synthesis, and, consequently, provide a therapeutic approach by which a disease can be treated by inhibiting the synthesis of proteins that contribute to the disease. The efficacy of antisense oligonucleotides for inhibiting protein synthesis is well established. For example, the synthesis of polygalactauronase and the muscarine type 2 acetylcholine receptor are inhibited by antisense oligonucleotides directed to their respective mRNA sequences (U. S. Patent 5,739,119 and U. S. Patent 5,759,829). Further, examples of antisense inhibition have been demonstrated with the nuclear protein cyclin, the multiple drug resistance gene (MDG1), ICAM-1, E-selectin, STK-1, striatal $GABA_A$ receptor and human EGF (Jaskulski *et al.*, Science. 1988 Jun 10;240(4858):1544-6; Vasanthakumar and Ahmed, Cancer Commun. 1989;1(4):225-32; Peris *et al.*, Brain Res Mol Brain Res. 1998 Jun 15;57(2):310-20; U. S. Patent 5,801,154; U.S. Patent 5,789,573; U. S. Patent 5,718,709 and U.S. Patent 5,610,288). Antisense constructs have also been described that inhibit and can be used to treat a variety of abnormal cellular proliferations, *e.g.* cancer (U. S. Patent 5,747,470; U. S. Patent 5,591,317 and U. S. Patent 5,783,683).

Therefore, in certain embodiments, the present invention provides oligonucleotide sequences that comprise all, or a portion of, any sequence that is capable of specifically binding to polynucleotide sequence described herein, or a complement thereof. In one embodiment, the antisense oligonucleotides comprise DNA or derivatives thereof. In another embodiment, the oligonucleotides comprise RNA or derivatives thereof. In a third embodiment, the oligonucleotides are modified DNAs comprising a phosphorothioated modified backbone. In a fourth embodiment, the oligonucleotide sequences comprise peptide nucleic acids or derivatives thereof. In each case, preferred compositions comprise a sequence region that is complementary, and more preferably substantially-complementary, and even more preferably, completely complementary to one or more portions of polynucleotides disclosed herein. Selection of antisense compositions specific for a given gene sequence is based upon analysis of the chosen target sequence and determination of secondary structure, T_m,

binding energy, and relative stability. Antisense compositions may be selected based upon their relative inability to form dimers, hairpins, or other secondary structures that would reduce or prohibit specific binding to the target mRNA in a host cell. Highly preferred target regions of the mRNA, are those which are at or near the AUG translation initiation codon, and those sequences which are substantially complementary to 5' regions of the mRNA. These secondary structure analyses and target site selection considerations can be performed, for example, using v.4 of the OLIGO primer analysis software and/or the BLASTN 2.0.5 algorithm software (Altschul *et al.,* Nucleic Acids Res. 1997, 25(17):3389-402).

The use of an antisense delivery method employing a short peptide vector, termed MPG (27 residues), is also contemplated. The MPG peptide contains a hydrophobic domain derived from the fusion sequence of HIV gp41 and a hydrophilic domain from the nuclear localization sequence of SV40 T-antigen (Morris *et al.*, Nucleic Acids Res. 1997 Jul 15;25(14):2730-6). It has been demonstrated that several molecules of the MPG peptide coat the antisense oligonucleotides and can be delivered into cultured mammalian cells in less than 1 hour with relatively high efficiency (90%). Further, the interaction with MPG strongly increases both the stability of the oligonucleotide to nuclease and the ability to cross the plasma membrane.

According to another embodiment of the invention, the polynucleotide compositions described herein are used in the design and preparation of ribozyme molecules for inhibiting expression of the tumor polypeptides and proteins of the present invention in tumor cells. Ribozymes are RNA-protein complexes that cleave nucleic acids in a site-specific fashion. Ribozymes have specific catalytic domains that possess endonuclease activity (Kim and Cech, Proc Natl Acad Sci U S A. 1987 Dec;84(24):8788-92; Forster and Symons, Cell. 1987 Apr 24;49(2):211-20). For example, a large number of ribozymes accelerate phosphoester transfer reactions with a high degree of specificity, often cleaving only one of several phosphoesters in an oligonucleotide substrate (Cech *et al.*, Cell. 1981 Dec;27(3 Pt 2):487-96; Michel and Westhof, J Mol Biol. 1990 Dec 5;216(3):585-610; Reinhold-Hurek and Shub, Nature. 1992 May 14;357(6374):173-6). This specificity has been attributed to the requirement

that the substrate bind via specific base-pairing interactions to the internal guide sequence ("IGS") of the ribozyme prior to chemical reaction.

Six basic varieties of naturally-occurring enzymatic RNAs are known presently. Each can catalyze the hydrolysis of RNA phosphodiester bonds *in trans* (and thus can cleave other RNA molecules) under physiological conditions. In general, enzymatic nucleic acids act by first binding to a target RNA. Such binding occurs through the target binding portion of a enzymatic nucleic acid which is held in close proximity to an enzymatic portion of the molecule that acts to cleave the target RNA. Thus, the enzymatic nucleic acid first recognizes and then binds a target RNA through complementary base-pairing, and once bound to the correct site, acts enzymatically to cut the target RNA. Strategic cleavage of such a target RNA will destroy its ability to direct synthesis of an encoded protein. After an enzymatic nucleic acid has bound and cleaved its RNA target, it is released from that RNA to search for another target and can repeatedly bind and cleave new targets.

The enzymatic nature of a ribozyme is advantageous over many technologies, such as antisense technology (where a nucleic acid molecule simply binds to a nucleic acid target to block its translation) since the concentration of ribozyme necessary to affect a therapeutic treatment is lower than that of an antisense oligonucleotide. This advantage reflects the ability of the ribozyme to act enzymatically. Thus, a single ribozyme molecule is able to cleave many molecules of target RNA. In addition, the ribozyme is a highly specific inhibitor, with the specificity of inhibition depending not only on the base pairing mechanism of binding to the target RNA, but also on the mechanism of target RNA cleavage. Single mismatches, or base-substitutions, near the site of cleavage can completely eliminate catalytic activity of a ribozyme. Similar mismatches in antisense molecules do not prevent their action (Woolf *et al.*, Proc Natl Acad Sci U S A. 1992 Aug 15;89(16):7305-9). Thus, the specificity of action of a ribozyme is greater than that of an antisense oligonucleotide binding the same RNA site.

The enzymatic nucleic acid molecule may be formed in a hammerhead, hairpin, a hepatitis δ virus, group I intron or RNaseP RNA (in association with an RNA guide sequence) or Neurospora VS RNA motif. Examples of hammerhead motifs are

described by Rossi *et al.* Nucleic Acids Res. 1992 Sep 11;20(17):4559-65. Examples of hairpin motifs are described by Hampel *et al.* (Eur. Pat. Appl. Publ. No. EP 0360257), Hampel and Tritz, Biochemistry 1989 Jun 13;28(12):4929-33; Hampel *et al.*, Nucleic Acids Res. 1990 Jan 25;18(2):299-304 and U. S. Patent 5,631,359. An example of the hepatitis δ virus motif is described by Perrotta and Been, Biochemistry. 1992 Dec 1;31(47):11843-52; an example of the RNaseP motif is described by Guerrier-Takada *et al.*, Cell. 1983 Dec;35(3 Pt 2):849-57; Neurospora VS RNA ribozyme motif is described by Collins (Saville and Collins, Cell. 1990 May 18;61(4):685-96; Saville and Collins, Proc Natl Acad Sci U S A. 1991 Oct 1;88(19):8826-30; Collins and Olive, Biochemistry. 1993 Mar 23;32(11):2795-9); and an example of the Group I intron is described in (U. S. Patent 4,987,071). All that is important in an enzymatic nucleic acid molecule of this invention is that it has a specific substrate binding site which is complementary to one or more of the target gene RNA regions, and that it have nucleotide sequences within or surrounding that substrate binding site which impart an RNA cleaving activity to the molecule. Thus the ribozyme constructs need not be limited to specific motifs mentioned herein.

Ribozymes may be designed as described in Int. Pat. Appl. Publ. No. WO 93/23569 and Int. Pat. Appl. Publ. No. WO 94/02595, each specifically incorporated herein by reference) and synthesized to be tested *in vitro* and *in vivo*, as described. Such ribozymes can also be optimized for delivery. While specific examples are provided, those in the art will recognize that equivalent RNA targets in other species can be utilized when necessary.

Ribozyme activity can be optimized by altering the length of the ribozyme binding arms, or chemically synthesizing ribozymes with modifications that prevent their degradation by serum ribonucleases (see *e.g.*, Int. Pat. Appl. Publ. No. WO 92/07065; Int. Pat. Appl. Publ. No. WO 93/15187; Int. Pat. Appl. Publ. No. WO 91/03162; Eur. Pat. Appl. Publ. No. 92110298.4; U. S. Patent 5,334,711; and Int. Pat. Appl. Publ. No. WO 94/13688, which describe various chemical modifications that can be made to the sugar moieties of enzymatic RNA molecules), modifications which enhance their efficacy in cells, and removal of stem II bases to shorten RNA synthesis times and reduce chemical requirements.

Sullivan *et al.* (Int. Pat. Appl. Publ. No. WO 94/02595) describes the general methods for delivery of enzymatic RNA molecules. Ribozymes may be administered to cells by a variety of methods known to those familiar to the art, including, but not restricted to, encapsulation in liposomes, by iontophoresis, or by incorporation into other vehicles, such as hydrogels, cyclodextrins, biodegradable nanocapsules, and bioadhesive microspheres. For some indications, ribozymes may be directly delivered *ex vivo* to cells or tissues with or without the aforementioned vehicles. Alternatively, the RNA/vehicle combination may be locally delivered by direct inhalation, by direct injection or by use of a catheter, infusion pump or stent. Other routes of delivery include, but are not limited to, intravascular, intramuscular, subcutaneous or joint injection, aerosol inhalation, oral (tablet or pill form), topical, systemic, ocular, intraperitoneal and/or intrathecal delivery. More detailed descriptions of ribozyme delivery and administration are provided in Int. Pat. Appl. Publ. No. WO 94/02595 and Int. Pat. Appl. Publ. No. WO 93/23569, each specifically incorporated herein by reference.

Another means of accumulating high concentrations of a ribozyme(s) within cells is to incorporate the ribozyme-encoding sequences into a DNA expression vector. Transcription of the ribozyme sequences are driven from a promoter for eukaryotic RNA polymerase I (pol I), RNA polymerase II (pol II), or RNA polymerase III (pol III). Transcripts from pol II or pol III promoters will be expressed at high levels in all cells; the levels of a given pol II promoter in a given cell type will depend on the nature of the gene regulatory sequences (enhancers, silencers, *etc.*) present nearby. Prokaryotic RNA polymerase promoters may also be used, providing that the prokaryotic RNA polymerase enzyme is expressed in the appropriate cells Ribozymes expressed from such promoters have been shown to function in mammalian cells. Such transcription units can be incorporated into a variety of vectors for introduction into mammalian cells, including but not restricted to, plasmid DNA vectors, viral DNA vectors (such as adenovirus or adeno-associated vectors), or viral RNA vectors (such as retroviral, semliki forest virus, sindbis virus vectors).

In another embodiment of the invention, peptide nucleic acids (PNAs) compositions are provided. PNA is a DNA mimic in which the nucleobases are

attached to a pseudopeptide backbone (Good and Nielsen, Antisense Nucleic Acid Drug Dev. 1997 7(4) 431-37). PNA is able to be utilized in a number methods that traditionally have used RNA or DNA. Often PNA sequences perform better in techniques than the corresponding RNA or DNA sequences and have utilities that are not inherent to RNA or DNA. A review of PNA including methods of making, characteristics of, and methods of using, is provided by Corey (*Trends Biotechnol* 1997 Jun;15(6):224-9). As such, in certain embodiments, one may prepare PNA sequences that are complementary to one or more portions of the ACE mRNA sequence, and such PNA compositions may be used to regulate, alter, decrease, or reduce the translation of ACE-specific mRNA, and thereby alter the level of ACE activity in a host cell to which such PNA compositions have been administered.

PNAs have 2-aminoethyl-glycine linkages replacing the normal phosphodiester backbone of DNA (Nielsen *et al.*, *Science* 1991 Dec 6;254(5037):1497-500; Hanvey *et al.*, Science. 1992 Nov 27;258(5087):1481-5; Hyrup and Nielsen, Bioorg Med Chem. 1996 Jan;4(1):5-23). This chemistry has three important consequences: firstly, in contrast to DNA or phosphorothioate oligonucleotides, PNAs are neutral molecules; secondly, PNAs are achiral, which avoids the need to develop a stereoselective synthesis; and thirdly, PNA synthesis uses standard Boc or Fmoc protocols for solid-phase peptide synthesis, although other methods, including a modified Merrifield method, have been used.

PNA monomers or ready-made oligomers are commercially available from PerSeptive Biosystems (Framingham, MA). PNA syntheses by either Boc or Fmoc protocols are straightforward using manual or automated protocols (Norton *et al.*, Bioorg Med Chem. 1995 Apr;3(4):437-45). The manual protocol lends itself to the production of chemically modified PNAs or the simultaneous synthesis of families of closely related PNAs.

As with peptide synthesis, the success of a particular PNA synthesis will depend on the properties of the chosen sequence. For example, while in theory PNAs can incorporate any combination of nucleotide bases, the presence of adjacent purines can lead to deletions of one or more residues in the product. In expectation of this difficulty, it is suggested that, in producing PNAs with adjacent purines, one should

repeat the coupling of residues likely to be added inefficiently. This should be followed by the purification of PNAs by reverse-phase high-pressure liquid chromatography, providing yields and purity of product similar to those observed during the synthesis of peptides.

Modifications of PNAs for a given application may be accomplished by coupling amino acids during solid-phase synthesis or by attaching compounds that contain a carboxylic acid group to the exposed N-terminal amine. Alternatively, PNAs can be modified after synthesis by coupling to an introduced lysine or cysteine. The ease with which PNAs can be modified facilitates optimization for better solubility or for specific functional requirements. Once synthesized, the identity of PNAs and their derivatives can be confirmed by mass spectrometry. Several studies have made and utilized modifications of PNAs (for example, Norton *et al.*, Bioorg Med Chem. 1995 Apr;3(4):437-45; Petersen *et al.*, J Pept Sci. 1995 May-Jun;1(3):175-83; Orum *et al.*, Biotechniques. 1995 Sep;19(3):472-80; Footer *et al.*, Biochemistry. 1996 Aug 20;35(33):10673-9; Griffith *et al.*, Nucleic Acids Res. 1995 Aug 11;23(15):3003-8; Pardridge *et al.*, Proc Natl Acad Sci U S A. 1995 Jun 6;92(12):5592-6; Boffa *et al.*, Proc Natl Acad Sci U S A. 1995 Mar 14;92(6):1901-5; Gambacorti-Passerini *et al.*, Blood. 1996 Aug 15;88(4):1411-7; Armitage *et al.*, Proc Natl Acad Sci U S A. 1997 Nov 11;94(23):12320-5; Seeger *et al.*, Biotechniques. 1997 Sep;23(3):512-7). U.S. Patent No. 5,700,922 discusses PNA-DNA-PNA chimeric molecules and their uses in diagnostics, modulating protein in organisms, and treatment of conditions susceptible to therapeutics.

Methods of characterizing the antisense binding properties of PNAs are discussed in Rose (Anal Chem. 1993 Dec 15;65(24):3545-9) and Jensen *et al.* (Biochemistry. 1997 Apr 22;36(16):5072-7). Rose uses capillary gel electrophoresis to determine binding of PNAs to their complementary oligonucleotide, measuring the relative binding kinetics and stoichiometry. Similar types of measurements were made by Jensen *et al.* using BIAcore™ technology.

Other applications of PNAs that have been described and will be apparent to the skilled artisan include use in DNA strand invasion, antisense inhibition, mutational analysis, enhancers of transcription, nucleic acid purification, isolation of

transcriptionally active genes, blocking of transcription factor binding, genome cleavage, biosensors, *in situ* hybridization, and the like.

POLYNUCLEOTIDE IDENTIFICATION, CHARACTERIZATION AND EXPRESSION

Polynucleotides compositions of the present invention may be identified, prepared and/or manipulated using any of a variety of well established techniques (see generally, Sambrook et al., *Molecular Cloning: A Laboratory Manual,* Cold Spring Harbor Laboratories, Cold Spring Harbor, NY, 1989, and other like references). For example, a polynucleotide may be identified, as described in more detail below, by screening a microarray of cDNAs for tumor-associated expression (*i.e.,* expression that is at least two fold greater in a tumor than in normal tissue, as determined using a representative assay provided herein). Such screens may be performed, for example, using the microarray technology of Affymetrix, Inc. (Santa Clara, CA) according to the manufacturer's instructions (and essentially as described by Schena et al., *Proc. Natl. Acad. Sci. USA 93*:10614-10619, 1996 and Heller et al., *Proc. Natl. Acad. Sci. USA 94*:2150-2155, 1997). Alternatively, polynucleotides may be amplified from cDNA prepared from cells expressing the proteins described herein, such as tumor cells.

Many template dependent processes are available to amplify a target sequences of interest present in a sample. One of the best known amplification methods is the polymerase chain reaction (PCR™) which is described in detail in U.S. Patent Nos. 4,683,195, 4,683,202 and 4,800,159, each of which is incorporated herein by reference in its entirety. Briefly, in PCR™, two primer sequences are prepared which are complementary to regions on opposite complementary strands of the target sequence. An excess of deoxynucleoside triphosphates is added to a reaction mixture along with a DNA polymerase (*e.g., Taq* polymerase). If the target sequence is present in a sample, the primers will bind to the target and the polymerase will cause the primers to be extended along the target sequence by adding on nucleotides. By raising and lowering the temperature of the reaction mixture, the extended primers will dissociate from the target to form reaction products, excess primers will bind to the target and to the reaction product and the process is repeated. Preferably reverse transcription and PCR™ amplification procedure may be performed in order to quantify

the amount of mRNA amplified. Polymerase chain reaction methodologies are well known in the art.

Any of a number of other template dependent processes, many of which are variations of the PCR ™ amplification technique, are readily known and available in the art. Illustratively, some such methods include the ligase chain reaction (referred to as LCR), described, for example, in Eur. Pat. Appl. Publ. No. 320,308 and U.S. Patent No. 4,883,750; Qbeta Replicase, described in PCT Intl. Pat. Appl. Publ. No. PCT/US87/00880; Strand Displacement Amplification (SDA) and Repair Chain Reaction (RCR). Still other amplification methods are described in Great Britain Pat. Appl. No. 2 202 328, and in PCT Intl. Pat. Appl. Publ. No. PCT/US89/01025. Other nucleic acid amplification procedures include transcription-based amplification systems (TAS) (PCT Intl. Pat. Appl. Publ. No. WO 88/10315), including nucleic acid sequence based amplification (NASBA) and 3SR. Eur. Pat. Appl. Publ. No. 329,822 describes a nucleic acid amplification process involving cyclically synthesizing single-stranded RNA ("ssRNA"), ssDNA, and double-stranded DNA (dsDNA). PCT Intl. Pat. Appl. Publ. No. WO 89/06700 describes a nucleic acid sequence amplification scheme based on the hybridization of a promoter/primer sequence to a target single-stranded DNA ("ssDNA") followed by transcription of many RNA copies of the sequence. Other amplification methods such as "RACE" (Frohman, 1990), and "one-sided PCR" (Ohara, 1989) are also well-known to those of skill in the art.

An amplified portion of a polynucleotide of the present invention may be used to isolate a full length gene from a suitable library (*e.g.*, a tumor cDNA library) using well known techniques. Within such techniques, a library (cDNA or genomic) is screened using one or more polynucleotide probes or primers suitable for amplification. Preferably, a library is size-selected to include larger molecules. Random primed libraries may also be preferred for identifying 5' and upstream regions of genes. Genomic libraries are preferred for obtaining introns and extending 5' sequences.

For hybridization techniques, a partial sequence may be labeled (*e.g.*, by nick-translation or end-labeling with ^{32}P) using well known techniques. A bacterial or bacteriophage library is then generally screened by hybridizing filters containing denatured bacterial colonies (or lawns containing phage plaques) with the labeled probe

(*see* Sambrook et al., *Molecular Cloning: A Laboratory Manual,* Cold Spring Harbor Laboratories, Cold Spring Harbor, NY, 1989). Hybridizing colonies or plaques are selected and expanded, and the DNA is isolated for further analysis. cDNA clones may be analyzed to determine the amount of additional sequence by, for example, PCR using a primer from the partial sequence and a primer from the vector. Restriction maps and partial sequences may be generated to identify one or more overlapping clones. The complete sequence may then be determined using standard techniques, which may involve generating a series of deletion clones. The resulting overlapping sequences can then assembled into a single contiguous sequence. A full length cDNA molecule can be generated by ligating suitable fragments, using well known techniques.

Alternatively, amplification techniques, such as those described above, can be useful for obtaining a full length coding sequence from a partial cDNA sequence. One such amplification technique is inverse PCR (*see* Triglia et al., *Nucl. Acids Res. 16*:8186, 1988), which uses restriction enzymes to generate a fragment in the known region of the gene. The fragment is then circularized by intramolecular ligation and used as a template for PCR with divergent primers derived from the known region. Within an alternative approach, sequences adjacent to a partial sequence may be retrieved by amplification with a primer to a linker sequence and a primer specific to a known region. The amplified sequences are typically subjected to a second round of amplification with the same linker primer and a second primer specific to the known region. A variation on this procedure, which employs two primers that initiate extension in opposite directions from the known sequence, is described in WO 96/38591. Another such technique is known as "rapid amplification of cDNA ends" or RACE. This technique involves the use of an internal primer and an external primer, which hybridizes to a polyA region or vector sequence, to identify sequences that are 5' and 3' of a known sequence. Additional techniques include capture PCR (Lagerstrom et al., *PCR Methods Applic. 1*:111-19, 1991) and walking PCR (Parker et al., *Nucl. Acids. Res. 19*:3055-60, 1991). Other methods employing amplification may also be employed to obtain a full length cDNA sequence.

In certain instances, it is possible to obtain a full length cDNA sequence by analysis of sequences provided in an expressed sequence tag (EST) database, such as

that available from GenBank. Searches for overlapping ESTs may generally be performed using well known programs (*e.g.*, NCBI BLAST searches), and such ESTs may be used to generate a contiguous full length sequence. Full length DNA sequences may also be obtained by analysis of genomic fragments.

In other embodiments of the invention, polynucleotide sequences or fragments thereof which encode polypeptides of the invention, or fusion proteins or functional equivalents thereof, may be used in recombinant DNA molecules to direct expression of a polypeptide in appropriate host cells. Due to the inherent degeneracy of the genetic code, other DNA sequences that encode substantially the same or a functionally equivalent amino acid sequence may be produced and these sequences may be used to clone and express a given polypeptide.

As will be understood by those of skill in the art, it may be advantageous in some instances to produce polypeptide-encoding nucleotide sequences possessing non-naturally occurring codons. For example, codons preferred by a particular prokaryotic or eukaryotic host can be selected to increase the rate of protein expression or to produce a recombinant RNA transcript having desirable properties, such as a half-life which is longer than that of a transcript generated from the naturally occurring sequence.

Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. In addition, site-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, or introduce mutations, and so forth.

In another embodiment of the invention, natural, modified, or recombinant nucleic acid sequences may be ligated to a heterologous sequence to encode a fusion protein. For example, to screen peptide libraries for inhibitors of polypeptide activity, it may be useful to encode a chimeric protein that can be

recognized by a commercially available antibody. A fusion protein may also be engineered to contain a cleavage site located between the polypeptide-encoding sequence and the heterologous protein sequence, so that the polypeptide may be cleaved and purified away from the heterologous moiety.

Sequences encoding a desired polypeptide may be synthesized, in whole or in part, using chemical methods well known in the art (see Caruthers, M. H. et al. (1980) *Nucl. Acids Res. Symp. Ser.* 215-223, Horn, T. et al. (1980) *Nucl. Acids Res. Symp. Ser.* 225-232). Alternatively, the protein itself may be produced using chemical methods to synthesize the amino acid sequence of a polypeptide, or a portion thereof. For example, peptide synthesis can be performed using various solid-phase techniques (Roberge, J. Y. et al. (1995) *Science 269*:202-204) and automated synthesis may be achieved, for example, using the ABI 431A Peptide Synthesizer (Perkin Elmer, Palo Alto, CA).

A newly synthesized peptide may be substantially purified by preparative high performance liquid chromatography (*e.g.*, Creighton, T. (1983) Proteins, Structures and Molecular Principles, WH Freeman and Co., New York, N.Y.) or other comparable techniques available in the art. The composition of the synthetic peptides may be confirmed by amino acid analysis or sequencing (*e.g.*, the Edman degradation procedure). Additionally, the amino acid sequence of a polypeptide, or any part thereof, may be altered during direct synthesis and/or combined using chemical methods with sequences from other proteins, or any part thereof, to produce a variant polypeptide.

In order to express a desired polypeptide, the nucleotide sequences encoding the polypeptide, or functional equivalents, may be inserted into appropriate expression vector, *i.e.*, a vector which contains the necessary elements for the transcription and translation of the inserted coding sequence. Methods which are well known to those skilled in the art may be used to construct expression vectors containing sequences encoding a polypeptide of interest and appropriate transcriptional and translational control elements. These methods include *in vitro* recombinant DNA techniques, synthetic techniques, and *in vivo* genetic recombination. Such techniques are described, for example, in Sambrook, J. et al. (1989) Molecular Cloning, A

Laboratory Manual, Cold Spring Harbor Press, Plainview, N.Y., and Ausubel, F. M. et al. (1989) Current Protocols in Molecular Biology, John Wiley & Sons, New York. N.Y.

A variety of expression vector/host systems may be utilized to contain and express polynucleotide sequences. These include, but are not limited to, microorganisms such as bacteria transformed with recombinant bacteriophage, plasmid, or cosmid DNA expression vectors; yeast transformed with yeast expression vectors; insect cell systems infected with virus expression vectors (*e.g.*, baculovirus); plant cell systems transformed with virus expression vectors (*e.g.*, cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or with bacterial expression vectors (*e.g.*, Ti or pBR322 plasmids); or animal cell systems.

The "control elements" or "regulatory sequences" present in an expression vector are those non-translated regions of the vector--enhancers, promoters, 5' and 3' untranslated regions--which interact with host cellular proteins to carry out transcription and translation. Such elements may vary in their strength and specificity. Depending on the vector system and host utilized, any number of suitable transcription and translation elements, including constitutive and inducible promoters, may be used. For example, when cloning in bacterial systems, inducible promoters such as the hybrid lacZ promoter of the pBLUESCRIPT phagemid (Stratagene, La Jolla, Calif.) or pSPORT1 plasmid (Gibco BRL, Gaithersburg, MD) and the like may be used. In mammalian cell systems, promoters from mammalian genes or from mammalian viruses are generally preferred. If it is necessary to generate a cell line that contains multiple copies of the sequence encoding a polypeptide, vectors based on SV40 or EBV may be advantageously used with an appropriate selectable marker.

In bacterial systems, any of a number of expression vectors may be selected depending upon the use intended for the expressed polypeptide. For example, when large quantities are needed, for example for the induction of antibodies, vectors which direct high level expression of fusion proteins that are readily purified may be used. Such vectors include, but are not limited to, the multifunctional *E. coli* cloning and expression vectors such as pBLUESCRIPT (Stratagene), in which the sequence encoding the polypeptide of interest may be ligated into the vector in frame with

sequences for the amino-terminal Met and the subsequent 7 residues of .beta.-galactosidase so that a hybrid protein is produced; pIN vectors (Van Heeke, G. and S. M. Schuster (1989) *J. Biol. Chem. 264*:5503-5509); and the like. pGEX Vectors (Promega, Madison, Wis.) may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. Proteins made in such systems may be designed to include heparin, thrombin, or factor XA protease cleavage sites so that the cloned polypeptide of interest can be released from the GST moiety at will.

In the yeast, Saccharomyces cerevisiae, a number of vectors containing constitutive or inducible promoters such as alpha factor, alcohol oxidase, and PGH may be used. For reviews, see Ausubel et al. (supra) and Grant et al. (1987) *Methods Enzymol.* 153:516-544.

In cases where plant expression vectors are used, the expression of sequences encoding polypeptides may be driven by any of a number of promoters. For example, viral promoters such as the 35S and 19S promoters of CaMV may be used alone or in combination with the omega leader sequence from TMV (Takamatsu, N. (1987) *EMBO J. 6*:307-311. Alternatively, plant promoters such as the small subunit of RUBISCO or heat shock promoters may be used (Coruzzi, G. et al. (1984) *EMBO J. 3*:1671-1680; Broglie, R. et al. (1984) *Science 224*:838-843; and Winter, J. et al. (1991) *Results Probl. Cell Differ. 17*:85-105). These constructs can be introduced into plant cells by direct DNA transformation or pathogen-mediated transfection. Such techniques are described in a number of generally available reviews (see, for example, Hobbs, S. or Murry, L. E. in McGraw Hill Yearbook of Science and Technology (1992) McGraw Hill, New York, N.Y.; pp. 191-196).

An insect system may also be used to express a polypeptide of interest. For example, in one such system, Autographa californica nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes in Spodoptera frugiperda cells or in Trichoplusia larvae. The sequences encoding the polypeptide may be cloned into a non-essential region of the virus, such as the polyhedrin gene, and placed under control

of the polyhedrin promoter. Successful insertion of the polypeptide-encoding sequence will render the polyhedrin gene inactive and produce recombinant virus lacking coat protein. The recombinant viruses may then be used to infect, for example, S. frugiperda cells or Trichoplusia larvae in which the polypeptide of interest may be expressed (Engelhard, E. K. et al. (1994) *Proc. Natl. Acad. Sci. 91* :3224-3227).

In mammalian host cells, a number of viral-based expression systems are generally available. For example, in cases where an adenovirus is used as an expression vector, sequences encoding a polypeptide of interest may be ligated into an adenovirus transcription/translation complex consisting of the late promoter and tripartite leader sequence. Insertion in a non-essential E1 or E3 region of the viral genome may be used to obtain a viable virus which is capable of expressing the polypeptide in infected host cells (Logan, J. and Shenk, T. (1984) *Proc. Natl. Acad. Sci. 81*:3655-3659). In addition, transcription enhancers, such as the Rous sarcoma virus (RSV) enhancer, may be used to increase expression in mammalian host cells.

Specific initiation signals may also be used to achieve more efficient translation of sequences encoding a polypeptide of interest. Such signals include the ATG initiation codon and adjacent sequences. In cases where sequences encoding the polypeptide, its initiation codon, and upstream sequences are inserted into the appropriate expression vector, no additional transcriptional or translational control signals may be needed. However, in cases where only coding sequence, or a portion thereof, is inserted, exogenous translational control signals including the ATG initiation codon should be provided. Furthermore, the initiation codon should be in the correct reading frame to ensure translation of the entire insert. Exogenous translational elements and initiation codons may be of various origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of enhancers which are appropriate for the particular cell system which is used, such as those described in the literature (Scharf, D. et al. (1994) *Results Probl. Cell Differ. 20*:125-162).

In addition, a host cell strain may be chosen for its ability to modulate the expression of the inserted sequences or to process the expressed protein in the desired fashion. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation. glycosylation, phosphorylation, lipidation, and acylation.

Post-translational processing which cleaves a "prepro" form of the protein may also be used to facilitate correct insertion, folding and/or function. Different host cells such as CHO, COS, HeLa, MDCK, HEK293, and WI38, which have specific cellular machinery and characteristic mechanisms for such post-translational activities, may be chosen to ensure the correct modification and processing of the foreign protein.

For long-term, high-yield production of recombinant proteins, stable expression is generally preferred. For example, cell lines which stably express a polynucleotide of interest may be transformed using expression vectors which may contain viral origins of replication and/or endogenous expression elements and a selectable marker gene on the same or on a separate vector. Following the introduction of the vector, cells may be allowed to grow for 1-2 days in an enriched media before they are switched to selective media. The purpose of the selectable marker is to confer resistance to selection, and its presence allows growth and recovery of cells which successfully express the introduced sequences. Resistant clones of stably transformed cells may be proliferated using tissue culture techniques appropriate to the cell type.

Any number of selection systems may be used to recover transformed cell lines. These include, but are not limited to, the herpes simplex virus thymidine kinase (Wigler, M. et al. (1977) *Cell 11*:223-32) and adenine phosphoribosyltransferase (Lowy, I. et al. (1990) *Cell 22*:817-23) genes which can be employed in tk.sup.- or aprt.sup.- cells, respectively. Also, antimetabolite, antibiotic or herbicide resistance can be used as the basis for selection; for example, dhfr which confers resistance to methotrexate (Wigler, M. et al. (1980) *Proc. Natl. Acad. Sci. 77*:3567-70); npt, which confers resistance to the aminoglycosides, neomycin and G-418 (Colbere-Garapin, F. et al (1981) *J. Mol. Biol. 150*:1-14); and als or pat, which confer resistance to chlorsulfuron and phosphinotricin acetyltransferase, respectively (Murry, *supra*). Additional selectable genes have been described, for example, trpB, which allows cells to utilize indole in place of tryptophan, or hisD, which allows cells to utilize histinol in place of histidine (Hartman, S. C. and R. C. Mulligan (1988) *Proc. Natl. Acad. Sci. 85*:8047-51). The use of visible markers has gained popularity with such markers as anthocyanins, beta-glucuronidase and its substrate GUS, and luciferase and its substrate luciferin, being widely used not only to identify transformants, but also to quantify the

amount of transient or stable protein expression attributable to a specific vector system (Rhodes, C. A. et al. (1995) *Methods Mol. Biol.* *55*:121-131).

Although the presence/absence of marker gene expression suggests that the gene of interest is also present, its presence and expression may need to be confirmed. For example, if the sequence encoding a polypeptide is inserted within a marker gene sequence, recombinant cells containing sequences can be identified by the absence of marker gene function. Alternatively, a marker gene can be placed in tandem with a polypeptide-encoding sequence under the control of a single promoter. Expression of the marker gene in response to induction or selection usually indicates expression of the tandem gene as well.

Alternatively, host cells that contain and express a desired polynucleotide sequence may be identified by a variety of procedures known to those of skill in the art. These procedures include, but are not limited to, DNA-DNA or DNA-RNA hybridizations and protein bioassay or immunoassay techniques which include, for example, membrane, solution, or chip based technologies for the detection and/or quantification of nucleic acid or protein.

A variety of protocols for detecting and measuring the expression of polynucleotide-encoded products, using either polyclonal or monoclonal antibodies specific for the product are known in the art. Examples include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and fluorescence activated cell sorting (FACS). A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes on a given polypeptide may be preferred for some applications, but a competitive binding assay may also be employed. These and other assays are described, among other places, in Hampton, R. et al. (1990; Serological Methods, a Laboratory Manual, APS Press, St Paul. Minn.) and Maddox, D. E. et al. (1983; *J. Exp. Med.* *158*:1211-1216).

A wide variety of labels and conjugation techniques are known by those skilled in the art and may be used in various nucleic acid and amino acid assays. Means for producing labeled hybridization or PCR probes for detecting sequences related to polynucleotides include oligolabeling, nick translation, end-labeling or PCR amplification using a labeled nucleotide. Alternatively, the sequences, or any portions

thereof may be cloned into a vector for the production of an mRNA probe. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA probes in vitro by addition of an appropriate RNA polymerase such as T7, T3, or SP6 and labeled nucleotides. These procedures may be conducted using a variety of commercially available kits. Suitable reporter molecules or labels, which may be used include radionuclides, enzymes, fluorescent, chemiluminescent, or chromogenic agents as well as substrates, cofactors, inhibitors, magnetic particles, and the like.

Host cells transformed with a polynucleotide sequence of interest may be cultured under conditions suitable for the expression and recovery of the protein from cell culture. The protein produced by a recombinant cell may be secreted or contained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing polynucleotides of the invention may be designed to contain signal sequences which direct secretion of the encoded polypeptide through a prokaryotic or eukaryotic cell membrane. Other recombinant constructions may be used to join sequences encoding a polypeptide of interest to nucleotide sequence encoding a polypeptide domain which will facilitate purification of soluble proteins. Such purification facilitating domains include, but are not limited to, metal chelating peptides such as histidine-tryptophan modules that allow purification on immobilized metals, protein A domains that allow purification on immobilized immunoglobulin, and the domain utilized in the FLAGS extension/affinity purification system (Immunex Corp., Seattle, Wash.). The inclusion of cleavable linker sequences such as those specific for Factor XA or enterokinase (Invitrogen. San Diego, Calif.) between the purification domain and the encoded polypeptide may be used to facilitate purification. One such expression vector provides for expression of a fusion protein containing a polypeptide of interest and a nucleic acid encoding 6 histidine residues preceding a thioredoxin or an enterokinase cleavage site. The histidine residues facilitate purification on IMIAC (immobilized metal ion affinity chromatography) as described in Porath, J. et al. (1992, *Prot. Exp. Purif. 3*:263-281) while the enterokinase cleavage site provides a means for purifying the desired polypeptide from the fusion protein. A discussion of vectors which contain fusion proteins is provided in Kroll, D. J. et al. (1993; *DNA Cell Biol. 12*:441-453).

In addition to recombinant production methods, polypeptides of the invention, and fragments thereof, may be produced by direct peptide synthesis using solid-phase techniques (Merrifield J. (1963) *J. Am. Chem. Soc. 85*:2149-2154). Protein synthesis may be performed using manual techniques or by automation. Automated synthesis may be achieved, for example, using Applied Biosystems 431A Peptide Synthesizer (Perkin Elmer). Alternatively, various fragments may be chemically synthesized separately and combined using chemical methods to produce the full length molecule.

ANTIBODY COMPOSITIONS, FRAGMENTS THEREOF AND OTHER BINDING AGENTS

According to another aspect, the present invention further provides binding agents, such as antibodies and antigen-binding fragments thereof, that exhibit immunological binding to a tumor polypeptide disclosed herein, or to a portion, variant or derivative thereof. An antibody, or antigen-binding fragment thereof, is said to "specifically bind," "immunogically bind," and/or is "immunologically reactive" to a polypeptide of the invention if it reacts at a detectable level (within, for example, an ELISA assay) with the polypeptide, and does not react detectably with unrelated polypeptides under similar conditions.

Immunological binding, as used in this context, generally refers to the non-covalent interactions of the type which occur between an immunoglobulin molecule and an antigen for which the immunoglobulin is specific. The strength, or affinity of immunological binding interactions can be expressed in terms of the dissociation constant (K_d) of the interaction, wherein a smaller K_d represents a greater affinity. Immunological binding properties of selected polypeptides can be quantified using methods well known in the art. One such method entails measuring the rates of antigen-binding site/antigen complex formation and dissociation, wherein those rates depend on the concentrations of the complex partners, the affinity of the interaction, and on geometric parameters that equally influence the rate in both directions. Thus, both the "on rate constant" (K_{on}) and the "off rate constant" (K_{off}) can be determined by calculation of the concentrations and the actual rates of association and dissociation. The ratio of K_{off}/K_{on} enables cancellation of all parameters not related to affinity, and is

thus equal to the dissociation constant K_d. See, generally, Davies et al. (1990) Annual Rev. Biochem. 59:439-473.

An "antigen-binding site," or "binding portion" of an antibody refers to the part of the immunoglobulin molecule that participates in antigen binding. The antigen binding site is formed by amino acid residues of the N-terminal variable ("V") regions of the heavy ("H") and light ("L") chains. Three highly divergent stretches within the V regions of the heavy and light chains are referred to as "hypervariable regions" which are interposed between more conserved flanking stretches known as "framework regions," or "FRs". Thus the term "FR" refers to amino acid sequences which are naturally found between and adjacent to hypervariable regions in immunoglobulins. In an antibody molecule, the three hypervariable regions of a light chain and the three hypervariable regions of a heavy chain are disposed relative to each other in three dimensional space to form an antigen-binding surface. The antigen-binding surface is complementary to the three-dimensional surface of a bound antigen, and the three hypervariable regions of each of the heavy and light chains are referred to as "complementarity-determining regions," or "CDRs."

Binding agents may be further capable of differentiating between patients with and without a cancer, such as colon cancer, using the representative assays provided herein. For example, antibodies or other binding agents that bind to a tumor protein will preferably generate a signal indicating the presence of a cancer in at least about 20% of patients with the disease, more preferably at least about 30% of patients. Alternatively, or in addition, the antibody will generate a negative signal indicating the absence of the disease in at least about 90% of individuals without the cancer. To determine whether a binding agent satisfies this requirement, biological samples (*e.g.*, blood, sera, sputum, urine and/or tumor biopsies) from patients with and without a cancer (as determined using standard clinical tests) may be assayed as described herein for the presence of polypeptides that bind to the binding agent. Preferably, a statistically significant number of samples with and without the disease will be assayed. Each binding agent should satisfy the above criteria; however, those of ordinary skill in the art will recognize that binding agents may be used in combination to improve sensitivity.

Any agent that satisfies the above requirements may be a binding agent. For example, a binding agent may be a ribosome, with or without a peptide component, an RNA molecule or a polypeptide. In a preferred embodiment, a binding agent is an antibody or an antigen-binding fragment thereof. Antibodies may be prepared by any of a variety of techniques known to those of ordinary skill in the art. *See, e.g.*, Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988. In general, antibodies can be produced by cell culture techniques, including the generation of monoclonal antibodies as described herein, or via transfection of antibody genes into suitable bacterial or mammalian cell hosts, in order to allow for the production of recombinant antibodies. In one technique, an immunogen comprising the polypeptide is initially injected into any of a wide variety of mammals (*e.g.*, mice, rats, rabbits, sheep or goats). In this step, the polypeptides of this invention may serve as the immunogen without modification. Alternatively, particularly for relatively short polypeptides, a superior immune response may be elicited if the polypeptide is joined to a carrier protein, such as bovine serum albumin or keyhole limpet hemocyanin. The immunogen is injected into the animal host, preferably according to a predetermined schedule incorporating one or more booster immunizations, and the animals are bled periodically. Polyclonal antibodies specific for the polypeptide may then be purified from such antisera by, for example, affinity chromatography using the polypeptide coupled to a suitable solid support.

Monoclonal antibodies specific for an antigenic polypeptide of interest may be prepared, for example, using the technique of Kohler and Milstein, *Eur. J. Immunol.* *6*:511-519, 1976, and improvements thereto. Briefly, these methods involve the preparation of immortal cell lines capable of producing antibodies having the desired specificity (*i.e.*, reactivity with the polypeptide of interest). Such cell lines may be produced, for example, from spleen cells obtained from an animal immunized as described above. The spleen cells are then immortalized by, for example, fusion with a myeloma cell fusion partner, preferably one that is syngeneic with the immunized animal. A variety of fusion techniques may be employed. For example, the spleen cells and myeloma cells may be combined with a nonionic detergent for a few minutes and then plated at low density on a selective medium that supports the growth of hybrid

cells, but not myeloma cells. A preferred selection technique uses HAT (hypoxanthine, aminopterin, thymidine) selection. After a sufficient time, usually about 1 to 2 weeks, colonies of hybrids are observed. Single colonies are selected and their culture supernatants tested for binding activity against the polypeptide. Hybridomas having high reactivity and specificity are preferred.

Monoclonal antibodies may be isolated from the supernatants of growing hybridoma colonies. In addition, various techniques may be employed to enhance the yield, such as injection of the hybridoma cell line into the peritoneal cavity of a suitable vertebrate host, such as a mouse. Monoclonal antibodies may then be harvested from the ascites fluid or the blood. Contaminants may be removed from the antibodies by conventional techniques, such as chromatography, gel filtration, precipitation, and extraction. The polypeptides of this invention may be used in the purification process in, for example, an affinity chromatography step.

A number of therapeutically useful molecules are known in the art which comprise antigen-binding sites that are capable of exhibiting immunological binding properties of an antibody molecule. The proteolytic enzyme papain preferentially cleaves IgG molecules to yield several fragments, two of which (the "F(ab)" fragments) each comprise a covalent heterodimer that includes an intact antigen-binding site. The enzyme pepsin is able to cleave IgG molecules to provide several fragments, including the "$F(ab')_2$ " fragment which comprises both antigen-binding sites. An "Fv" fragment can be produced by preferential proteolytic cleavage of an IgM, and on rare occasions IgG or IgA immunoglobulin molecule. Fv fragments are, however, more commonly derived using recombinant techniques known in the art. The Fv fragment includes a non-covalent $V_H::V_L$ heterodimer including an antigen-binding site which retains much of the antigen recognition and binding capabilities of the native antibody molecule. Inbar et al. (1972) Proc. Nat. Acad. Sci. USA 69:2659-2662; Hochman et al. (1976) Biochem 15:2706-2710; and Ehrlich et al. (1980) Biochem 19:4091-4096.

A single chain Fv ("sFv") polypeptide is a covalently linked $V_H::V_L$ heterodimer which is expressed from a gene fusion including V_H- and V_L-encoding genes linked by a peptide-encoding linker. Huston et al. (1988) Proc. Nat. Acad. Sci. USA 85(16):5879-5883. A number of methods have been described to discern chemical

structures for converting the naturally aggregated--but chemically separated--light and heavy polypeptide chains from an antibody V region into an sFv molecule which will fold into a three dimensional structure substantially similar to the structure of an antigen-binding site. See, *e.g.*, U.S. Pat. Nos. 5,091,513 and 5,132,405, to Huston et al.; and U.S. Pat. No. 4,946,778, to Ladner et al.

Each of the above-described molecules includes a heavy chain and a light chain CDR set, respectively interposed between a heavy chain and a light chain FR set which provide support to the CDRS and define the spatial relationship of the CDRs relative to each other. As used herein, the term "CDR set" refers to the three hypervariable regions of a heavy or light chain V region. Proceeding from the N-terminus of a heavy or light chain, these regions are denoted as "CDR1," "CDR2," and "CDR3" respectively. An antigen-binding site, therefore, includes six CDRs, comprising the CDR set from each of a heavy and a light chain V region. A polypeptide comprising a single CDR, (*e.g.*, a CDR1, CDR2 or CDR3) is referred to herein as a "molecular recognition unit." Crystallographic analysis of a number of antigen-antibody complexes has demonstrated that the amino acid residues of CDRs form extensive contact with bound antigen, wherein the most extensive antigen contact is with the heavy chain CDR3. Thus, the molecular recognition units are primarily responsible for the specificity of an antigen-binding site.

As used herein, the term "FR set" refers to the four flanking amino acid sequences which frame the CDRs of a CDR set of a heavy or light chain V region. Some FR residues may contact bound antigen; however, FRs are primarily responsible for folding the V region into the antigen-binding site, particularly the FR residues directly adjacent to the CDRS. Within FRs, certain amino residues and certain structural features are very highly conserved. In this regard, all V region sequences contain an internal disulfide loop of around 90 amino acid residues. When the V regions fold into a binding-site, the CDRs are displayed as projecting loop motifs which form an antigen-binding surface. It is generally recognized that there are conserved structural regions of FRs which influence the folded shape of the CDR loops into certain "canonical" structures--regardless of the precise CDR amino acid sequence. Further, certain FR

residues are known to participate in non-covalent interdomain contacts which stabilize the interaction of the antibody heavy and light chains.

A number of "humanized" antibody molecules comprising an antigen-binding site derived from a non-human immunoglobulin have been described, including chimeric antibodies having rodent V regions and their associated CDRs fused to human constant domains (Winter et al. (1991) Nature 349:293-299; Lobuglio et al. (1989) Proc. Nat. Acad. Sci. USA 86:4220-4224; Shaw et al. (1987) J Immunol. 138:4534-4538; and Brown et al. (1987) Cancer Res. 47:3577-3583), rodent CDRs grafted into a human supporting FR prior to fusion with an appropriate human antibody constant domain (Riechmann et al. (1988) Nature 332:323-327; Verhoeyen et al. (1988) Science 239:1534-1536; and Jones et al. (1986) Nature 321:522-525), and rodent CDRs supported by recombinantly veneered rodent FRs (European Patent Publication No. 519,596, published Dec. 23, 1992). These "humanized" molecules are designed to minimize unwanted immunological response toward rodent antihuman antibody molecules which limits the duration and effectiveness of therapeutic applications of those moieties in human recipients.

As used herein, the terms "veneered FRs" and "recombinantly veneered FRs" refer to the selective replacement of FR residues from, *e.g.*, a rodent heavy or light chain V region, with human FR residues in order to provide a xenogeneic molecule comprising an antigen-binding site which retains substantially all of the native FR polypeptide folding structure. Veneering techniques are based on the understanding that the ligand binding characteristics of an antigen-binding site are determined primarily by the structure and relative disposition of the heavy and light chain CDR sets within the antigen-binding surface. Davies et al. (1990) Ann. Rev. Biochem. 59:439-473. Thus, antigen binding specificity can be preserved in a humanized antibody only wherein the CDR structures, their interaction with each other, and their interaction with the rest of the V region domains are carefully maintained. By using veneering techniques, exterior (*e.g.*, solvent-accessible) FR residues which are readily encountered by the immune system are selectively replaced with human residues to provide a hybrid molecule that comprises either a weakly immunogenic, or substantially non-immunogenic veneered surface.

The process of veneering makes use of the available sequence data for human antibody variable domains compiled by Kabat et al., in Sequences of Proteins of Immunological Interest, 4th ed., (U.S. Dept. of Health and Human Services, U.S. Government Printing Office, 1987), updates to the Kabat database, and other accessible U.S. and foreign databases (both nucleic acid and protein). Solvent accessibilities of V region amino acids can be deduced from the known three-dimensional structure for human and murine antibody fragments. There are two general steps in veneering a murine antigen-binding site. Initially, the FRs of the variable domains of an antibody molecule of interest are compared with corresponding FR sequences of human variable domains obtained from the above-identified sources. The most homologous human V regions are then compared residue by residue to corresponding murine amino acids. The residues in the murine FR which differ from the human counterpart are replaced by the residues present in the human moiety using recombinant techniques well known in the art. Residue switching is only carried out with moieties which are at least partially exposed (solvent accessible), and care is exercised in the replacement of amino acid residues which may have a significant effect on the tertiary structure of V region domains, such as proline, glycine and charged amino acids.

In this manner, the resultant "veneered" murine antigen-binding sites are thus designed to retain the murine CDR residues, the residues substantially adjacent to the CDRs, the residues identified as buried or mostly buried (solvent inaccessible), the residues believed to participate in non-covalent (*e.g.*, electrostatic and hydrophobic) contacts between heavy and light chain domains, and the residues from conserved structural regions of the FRs which are believed to influence the "canonical" tertiary structures of the CDR loops. These design criteria are then used to prepare recombinant nucleotide sequences which combine the CDRs of both the heavy and light chain of a murine antigen-binding site into human-appearing FRs that can be used to transfect mammalian cells for the expression of recombinant human antibodies which exhibit the antigen specificity of the murine antibody molecule.

In another embodiment of the invention, monoclonal antibodies of the present invention may be coupled to one or more therapeutic agents. Suitable agents in this regard include radionuclides, differentiation inducers, drugs, toxins, and derivatives

thereof. Preferred radionuclides include ^{90}Y, ^{123}I, ^{125}I, ^{131}I, ^{186}Re, ^{188}Re, ^{211}At, and ^{212}Bi. Preferred drugs include methotrexate, and pyrimidine and purine analogs. Preferred differentiation inducers include phorbol esters and butyric acid. Preferred toxins include ricin, abrin, diptheria toxin, cholera toxin, gelonin, Pseudomonas exotoxin, Shigella toxin, and pokeweed antiviral protein.

A therapeutic agent may be coupled (*e.g.*, covalently bonded) to a suitable monoclonal antibody either directly or indirectly (*e.g.*, via a linker group). A direct reaction between an agent and an antibody is possible when each possesses a substituent capable of reacting with the other. For example, a nucleophilic group, such as an amino or sulfhydryl group, on one may be capable of reacting with a carbonyl-containing group, such as an anhydride or an acid halide, or with an alkyl group containing a good leaving group (*e.g.*, a halide) on the other.

Alternatively, it may be desirable to couple a therapeutic agent and an antibody via a linker group. A linker group can function as a spacer to distance an antibody from an agent in order to avoid interference with binding capabilities. A linker group can also serve to increase the chemical reactivity of a substituent on an agent or an antibody, and thus increase the coupling efficiency. An increase in chemical reactivity may also facilitate the use of agents, or functional groups on agents, which otherwise would not be possible.

It will be evident to those skilled in the art that a variety of bifunctional or polyfunctional reagents, both homo- and hetero-functional (such as those described in the catalog of the Pierce Chemical Co., Rockford, IL), may be employed as the linker group. Coupling may be effected, for example, through amino groups, carboxyl groups, sulfhydryl groups or oxidized carbohydrate residues. There are numerous references describing such methodology, *e.g.*, U.S. Patent No. 4,671,958, to Rodwell et al.

Where a therapeutic agent is more potent when free from the antibody portion of the immunoconjugates of the present invention, it may be desirable to use a linker group which is cleavable during or upon internalization into a cell. A number of different cleavable linker groups have been described. The mechanisms for the intracellular release of an agent from these linker groups include cleavage by reduction of a disulfide bond (*e.g.*, U.S. Patent No. 4,489,710, to Spitler), by irradiation of a

photolabile bond (*e.g.*, U.S. Patent No. 4,625,014, to Senter et al.), by hydrolysis of derivatized amino acid side chains (*e.g.*, U.S. Patent No. 4,638,045, to Kohn et al.), by serum complement-mediated hydrolysis (*e.g.*, U.S. Patent No. 4,671,958, to Rodwell et al.), and acid-catalyzed hydrolysis (*e.g.*, U.S. Patent No. 4,569,789, to Blattler et al.).

It may be desirable to couple more than one agent to an antibody. In one embodiment, multiple molecules of an agent are coupled to one antibody molecule. In another embodiment, more than one type of agent may be coupled to one antibody. Regardless of the particular embodiment, immunoconjugates with more than one agent may be prepared in a variety of ways. For example, more than one agent may be coupled directly to an antibody molecule, or linkers that provide multiple sites for attachment can be used. Alternatively, a carrier can be used.

A carrier may bear the agents in a variety of ways, including covalent bonding either directly or via a linker group. Suitable carriers include proteins such as albumins (*e.g.*, U.S. Patent No. 4,507,234, to Kato et al.), peptides and polysaccharides such as aminodextran (*e.g.*, U.S. Patent No. 4,699,784, to Shih et al.). A carrier may also bear an agent by noncovalent bonding or by encapsulation, such as within a liposome vesicle (*e.g.*, U.S. Patent Nos. 4,429,008 and 4,873,088). Carriers specific for radionuclide agents include radiohalogenated small molecules and chelating compounds. For example, U.S. Patent No. 4,735,792 discloses representative radiohalogenated small molecules and their synthesis. A radionuclide chelate may be formed from chelating compounds that include those containing nitrogen and sulfur atoms as the donor atoms for binding the metal, or metal oxide, radionuclide. For example, U.S. Patent No. 4,673,562, to Davison et al. discloses representative chelating compounds and their synthesis.

T CELL COMPOSITIONS

The present invention, in another aspect, provides T cells specific for a tumor polypeptide disclosed herein, or for a variant or derivative thereof. Such cells may generally be prepared *in vitro* or *ex vivo*, using standard procedures. For example, T cells may be isolated from bone marrow, peripheral blood, or a fraction of bone marrow or peripheral blood of a patient, using a commercially available cell separation

system, such as the Isolex™ System, available from Nexell Therapeutics, Inc. (Irvine, CA; see also U.S. Patent No. 5,240,856; U.S. Patent No. 5,215,926; WO 89/06280; WO 91/16116 and WO 92/07243). Alternatively, T cells may be derived from related or unrelated humans, non-human mammals, cell lines or cultures.

T cells may be stimulated with a polypeptide, polynucleotide encoding a polypeptide and/or an antigen presenting cell (APC) that expresses such a polypeptide. Such stimulation is performed under conditions and for a time sufficient to permit the generation of T cells that are specific for the polypeptide of interest. Preferably, a tumor polypeptide or polynucleotide of the invention is present within a delivery vehicle, such as a microsphere, to facilitate the generation of specific T cells.

T cells are considered to be specific for a polypeptide of the present invention if the T cells specifically proliferate, secrete cytokines or kill target cells coated with the polypeptide or expressing a gene encoding the polypeptide. T cell specificity may be evaluated using any of a variety of standard techniques. For example, within a chromium release assay or proliferation assay, a stimulation index of more than two fold increase in lysis and/or proliferation, compared to negative controls, indicates T cell specificity. Such assays may be performed, for example, as described in Chen et al., *Cancer Res. 54*:1065-1070, 1994. Alternatively, detection of the proliferation of T cells may be accomplished by a variety of known techniques. For example, T cell proliferation can be detected by measuring an increased rate of DNA synthesis (*e.g.*, by pulse-labeling cultures of T cells with tritiated thymidine and measuring the amount of tritiated thymidine incorporated into DNA). Contact with a tumor polypeptide (100 ng/ml - 100 μg/ml, preferably 200 ng/ml - 25 μg/ml) for 3 - 7 days will typically result in at least a two fold increase in proliferation of the T cells. Contact as described above for 2-3 hours should result in activation of the T cells, as measured using standard cytokine assays in which a two fold increase in the level of cytokine release (*e.g.*, TNF or IFN-γ) is indicative of T cell activation (*see* Coligan et al., Current Protocols in Immunology, vol. 1, Wiley Interscience (Greene 1998)). T cells that have been activated in response to a tumor polypeptide, polynucleotide or polypeptide-expressing APC may be $CD4^+$ and/or $CD8^+$. Tumor polypeptide-specific T cells may be expanded using standard techniques. Within preferred embodiments, the T

cells are derived from a patient, a related donor or an unrelated donor, and are administered to the patient following stimulation and expansion.

For therapeutic purposes, $CD4^{+}$ or $CD8^{+}$ T cells that proliferate in response to a tumor polypeptide, polynucleotide or APC can be expanded in number either *in vitro* or *in vivo*. Proliferation of such T cells *in vitro* may be accomplished in a variety of ways. For example, the T cells can be re-exposed to a tumor polypeptide, or a short peptide corresponding to an immunogenic portion of such a polypeptide, with or without the addition of T cell growth factors, such as interleukin-2, and/or stimulator cells that synthesize a tumor polypeptide. Alternatively, one or more T cells that proliferate in the presence of the tumor polypeptide can be expanded in number by cloning. Methods for cloning cells are well known in the art, and include limiting dilution.

T CELL RECEPTOR COMPOSITIONS

The T cell receptor (TCR) consists of 2 different, highly variable polypeptide chains, termed the T-cell receptor α and β chains, that are linked by a disulfide bond (Janeway, Travers, Walport. *Immunobiology.* Fourth Ed., 148-159. Elsevier Science Ltd/Garland Publishing. 1999). The α/β heterodimer complexes with the invariant CD3 chains at the cell membrane. This complex recognizes specific antigenic peptides bound to MHC molecules. The enormous diversity of TCR specificities is generated much like immunoglobulin diversity, through somatic gene rearrangement. The β chain genes contain over 50 variable (V), 2 diversity (D), over 10 joining (J) segments, and 2 constant region segments (C). The α chain genes contain over 70 V segments, and over 60 J segments but no D segments, as well as one C segment. During T cell development in the thymus, the D to J gene rearrangement of the β chain occurs, followed by the V gene segment rearrangement to the DJ. This functional VDJ_{β} exon is transcribed and spliced to join to a C_{β}. For the α chain, a V_{α} gene segment rearranges to a J_{α} gene segment to create the functional exon that is then transcribed and spliced to the C_{α}. Diversity is further increased during the recombination process by the random addition of P and N-nucleotides between the V, D, and J segments of the β chain and between the V and J segments in the α chain

(Janeway, Travers, Walport. *Immunobiology.* Fourth Ed., 98 and 150. Elsevier Science Ltd/Garland Publishing. 1999).

The present invention, in another aspect, provides TCRs specific for a colon tumor polypeptide disclosed herein, or for a variant or derivative thereof. In accordance with the present invention, polynucleotide and amino acid sequences are provided for the V-J or V-D-J junctional regions or parts thereof for the alpha and beta chains of the T-cell receptor which recognize tumor polypeptides described herein. In general, this aspect of the invention relates to T-cell receptors which recognize or bind tumor polypeptides presented in the context of MHC. In a preferred embodiment the tumor antigens recognized by the T-cell receptors comprise a polypeptide of the present invention. For example, cDNA encoding a TCR specific for a colon tumor peptide can be isolated from T cells specific for a tumor polypeptide using standard molecular biological and recombinant DNA techniques.

This invention further includes the T-cell receptors or analogs thereof having substantially the same function or activity as the T-cell receptors of this invention which recognize or bind tumor polypeptides. Such receptors include, but are not limited to, a fragment of the receptor, or a substitution, addition or deletion mutant of a T-cell receptor provided herein. This invention also encompasses polypeptides or peptides that are substantially homologous to the T-cell receptors provided herein or that retain substantially the same activity. The term "analog" includes any protein or polypeptide having an amino acid residue sequence substantially identical to the T-cell receptors provided herein in which one or more residues, preferably no more than 5 residues, more preferably no more than 25 residues have been conservatively substituted with a functionally similar residue and which displays the functional aspects of the T-cell receptor as described herein.

The present invention further provides for suitable mammalian host cells, for example, non-specific T cells, that are transfected with a polynucleotide encoding TCRs specific for a polypeptide described herein, thereby rendering the host cell specific for the polypeptide. The α and β chains of the TCR may be contained on separate expression vectors or alternatively, on a single expression vector that also contains an internal ribosome entry site (IRES) for cap-independent translation of the gene downstream of the IRES. Said host cells expressing TCRs specific for the

polypeptide may be used, for example, for adoptive immunotherapy of colon cancer as discussed further below.

In further aspects of the present invention, cloned TCRs specific for a polypeptide recited herein may be used in a kit for the diagnosis of colon cancer. For example, the nucleic acid sequence or portions thereof, of colon tumor-specific TCRs can be used as probes or primers for the detection of expression of the rearranged genes encoding the specific TCR in a biological sample. Therefore, the present invention further provides for an assay for detecting messenger RNA or DNA encoding the TCR specific for a polypeptide.

PHARMACEUTICAL COMPOSITIONS

In additional embodiments, the present invention concerns formulation of one or more of the polynucleotide, polypeptide, T-cell, TCR, and/or antibody compositions disclosed herein in pharmaceutically-acceptable carriers for administration to a cell or an animal, either alone, or in combination with one or more other modalities of therapy.

It will be understood that, if desired, a composition as disclosed herein may be administered in combination with other agents as well, such as, *e.g.*, other proteins or polypeptides or various pharmaceutically-active agents. In fact, there is virtually no limit to other components that may also be included, given that the additional agents do not cause a significant adverse effect upon contact with the target cells or host tissues. The compositions may thus be delivered along with various other agents as required in the particular instance. Such compositions may be purified from host cells or other biological sources, or alternatively may be chemically synthesized as described herein. Likewise, such compositions may further comprise substituted or derivatized RNA or DNA compositions.

Therefore, in another aspect of the present invention, pharmaceutical compositions are provided comprising one or more of the polynucleotide, polypeptide, antibody, TCR, and/or T-cell compositions described herein in combination with a physiologically acceptable carrier. In certain preferred embodiments, the pharmaceutical compositions of the invention comprise immunogenic polynucleotide

and/or polypeptide compositions of the invention for use in prophylactic and theraputic vaccine applications. Vaccine preparation is generally described in, for example, M.F. Powell and M.J. Newman, eds., "Vaccine Design (the subunit and adjuvant approach)," Plenum Press (NY, 1995). Generally, such compositions will comprise one or more polynucleotide and/or polypeptide compositions of the present invention in combination with one or more immunostimulants.

It will be apparent that any of the pharmaceutical compositions described herein can contain pharmaceutically acceptable salts of the polynucleotides and polypeptides of the invention. Such salts can be prepared, for example, from pharmaceutically acceptable non-toxic bases, including organic bases (*e.g.,* salts of primary, secondary and tertiary amines and basic amino acids) and inorganic bases (*e.g.,* sodium, potassium, lithium, ammonium, calcium and magnesium salts).

In another embodiment, illustrative immunogenic compositions, *e.g.*, vaccine compositions, of the present invention comprise DNA encoding one or more of the polypeptides as described above, such that the polypeptide is generated *in situ.* As noted above, the polynucleotide may be administered within any of a variety of delivery systems known to those of ordinary skill in the art. Indeed, numerous gene delivery techniques are well known in the art, such as those described by Rolland, *Crit. Rev. Therap. Drug Carrier Systems 15*:143-198, 1998, and references cited therein. Appropriate polynucleotide expression systems will, of course, contain the necessary regulatory DNA regulatory sequences for expression in a patient (such as a suitable promoter and terminating signal). Alternatively, bacterial delivery systems may involve the administration of a bacterium (such as *Bacillus-Calmette-Guerrin*) that expresses an immunogenic portion of the polypeptide on its cell surface or secretes such an epitope.

Therefore, in certain embodiments, polynucleotides encoding immunogenic polypeptides described herein are introduced into suitable mammalian host cells for expression using any of a number of known viral-based systems. In one illustrative embodiment, retroviruses provide a convenient and effective platform for gene delivery systems. A selected nucleotide sequence encoding a polypeptide of the present invention can be inserted into a vector and packaged in retroviral particles using techniques known in the art. The recombinant virus can then be isolated and delivered

to a subject. A number of illustrative retroviral systems have been described (*e.g.*, U.S. Pat. No. 5,219,740; Miller and Rosman (1989) BioTechniques 7:980-990; Miller, A. D. (1990) Human Gene Therapy 1:5-14; Scarpa et al. (1991) Virology 180:849-852; Burns et al. (1993) Proc. Natl. Acad. Sci. USA 90:8033-8037; and Boris-Lawrie and Temin (1993) Cur. Opin. Genet. Develop. 3:102-109.

In addition, a number of illustrative adenovirus-based systems have also been described. Unlike retroviruses which integrate into the host genome, adenoviruses persist extrachromosomally thus minimizing the risks associated with insertional mutagenesis (Haj-Ahmad and Graham (1986) J. Virol. 57:267-274; Bett et al. (1993) J. Virol. 67:5911-5921; Mittereder et al. (1994) Human Gene Therapy 5:717-729; Seth et al. (1994) J. Virol. 68:933-940; Barr et al. (1994) Gene Therapy 1:51-58; Berkner, K. L. (1988) BioTechniques 6:616-629; and Rich et al. (1993) Human Gene Therapy 4:461-476).

Various adeno-associated virus (AAV) vector systems have also been developed for polynucleotide delivery. AAV vectors can be readily constructed using techniques well known in the art. See, *e.g.*, U.S. Pat. Nos. 5,173,414 and 5,139,941; International Publication Nos. WO 92/01070 and WO 93/03769; Lebkowski et al. (1988) Molec. Cell. Biol. 8:3988-3996; Vincent et al. (1990) Vaccines 90 (Cold Spring Harbor Laboratory Press); Carter, B. J. (1992) Current Opinion in Biotechnology 3:533-539; Muzyczka, N. (1992) Current Topics in Microbiol. and Immunol. 158:97-129; Kotin, R. M. (1994) Human Gene Therapy 5:793-801; Shelling and Smith (1994) Gene Therapy 1:165-169; and Zhou et al. (1994) J. Exp. Med. 179:1867-1875.

Additional viral vectors useful for delivering the polynucleotides encoding polypeptides of the present invention by gene transfer include those derived from the pox family of viruses, such as vaccinia virus and avian poxvirus. By way of example, vaccinia virus recombinants expressing the novel molecules can be constructed as follows. The DNA encoding a polypeptide is first inserted into an appropriate vector so that it is adjacent to a vaccinia promoter and flanking vaccinia DNA sequences, such as the sequence encoding thymidine kinase (TK). This vector is then used to transfect cells which are simultaneously infected with vaccinia. Homologous recombination serves to insert the vaccinia promoter plus the gene

encoding the polypeptide of interest into the viral genome. The resulting TK.sup.(-) recombinant can be selected by culturing the cells in the presence of 5-bromodeoxyuridine and picking viral plaques resistant thereto.

A vaccinia-based infection/transfection system can be conveniently used to provide for inducible, transient expression or coexpression of one or more polypeptides described herein in host cells of an organism. In this particular system, cells are first infected in vitro with a vaccinia virus recombinant that encodes the bacteriophage T7 RNA polymerase. This polymerase displays exquisite specificity in that it only transcribes templates bearing T7 promoters. Following infection, cells are transfected with the polynucleotide or polynucleotides of interest, driven by a T7 promoter. The polymerase expressed in the cytoplasm from the vaccinia virus recombinant transcribes the transfected DNA into RNA which is then translated into polypeptide by the host translational machinery. The method provides for high level, transient, cytoplasmic production of large quantities of RNA and its translation products. See, *e.g.*, Elroy-Stein and Moss, Proc. Natl. Acad. Sci. USA (1990) 87:6743-6747; Fuerst et al. Proc. Natl. Acad. Sci. USA (1986) 83:8122-8126.

Alternatively, avipoxviruses, such as the fowlpox and canarypox viruses, can also be used to deliver the coding sequences of interest. Recombinant avipox viruses, expressing immunogens from mammalian pathogens, are known to confer protective immunity when administered to non-avian species. The use of an Avipox vector is particularly desirable in human and other mammalian species since members of the Avipox genus can only productively replicate in susceptible avian species and therefore are not infective in mammalian cells. Methods for producing recombinant Avipoxviruses are known in the art and employ genetic recombination, as described above with respect to the production of vaccinia viruses. See, *e.g.*, WO 91/12882; WO 89/03429; and WO 92/03545.

Any of a number of alphavirus vectors can also be used for delivery of polynucleotide compositions of the present invention, such as those vectors described in U.S. Patent Nos. 5,843,723; 6,015,686; 6,008,035 and 6,015,694. Certain vectors based on Venezuelan Equine Encephalitis (VEE) can also be used, illustrative examples of which can be found in U.S. Patent Nos. 5,505,947 and 5,643,576.

Moreover, molecular conjugate vectors, such as the adenovirus chimeric vectors described in Michael et al. J. Biol. Chem. (1993) 268:6866-6869 and Wagner et al. Proc. Natl. Acad. Sci. USA (1992) 89:6099-6103, can also be used for gene delivery under the invention.

Additional illustrative information on these and other known viral-based delivery systems can be found, for example, in Fisher-Hoch et al., *Proc. Natl. Acad. Sci. USA 86*:317-321, 1989; Flexner et al., *Ann. N.Y. Acad. Sci. 569*:86-103, 1989; Flexner et al., *Vaccine 8*:17-21, 1990; U.S. Patent Nos. 4,603,112, 4,769,330, and 5,017,487; WO 89/01973; U.S. Patent No. 4,777,127; GB 2,200,651; EP 0,345,242; WO 91/02805; Berkner, *Biotechniques 6*:616-627, 1988; Rosenfeld et al., *Science 252*:431-434, 1991; Kolls et al., *Proc. Natl. Acad. Sci. USA 91*:215-219, 1994; Kass-Eisler et al., *Proc. Natl. Acad. Sci. USA 90*:11498-11502, 1993; Guzman et al., *Circulation 88*:2838-2848, 1993; and Guzman et al., *Cir. Res. 73*:1202-1207, 1993.

In certain embodiments, a polynucleotide may be integrated into the genome of a target cell. This integration may be in the specific location and orientation *via* homologous recombination (gene replacement) or it may be integrated in a random, non-specific location (gene augmentation). In yet further embodiments, the polynucleotide may be stably maintained in the cell as a separate, episomal segment of DNA. Such polynucleotide segments or "episomes" encode sequences sufficient to permit maintenance and replication independent of or in synchronization with the host cell cycle. The manner in which the expression construct is delivered to a cell and where in the cell the polynucleotide remains is dependent on the type of expression construct employed.

In another embodiment of the invention, a polynucleotide is administered/delivered as "naked" DNA, for example as described in Ulmer et al., *Science 259*:1745-1749, 1993 and reviewed by Cohen, *Science 259*:1691-1692, 1993. The uptake of naked DNA may be increased by coating the DNA onto biodegradable beads, which are efficiently transported into the cells.

In still another embodiment, a composition of the present invention can be delivered via a particle bombardment approach, many of which have been described. In one illustrative example, gas-driven particle acceleration can be achieved with

devices such as those manufactured by Powderject Pharmaceuticals PLC (Oxford, UK) and Powderject Vaccines Inc. (Madison, WI), some examples of which are described in U.S. Patent Nos. 5,846,796; 6,010,478; 5,865,796; 5,584,807; and EP Patent No. 0500 799. This approach offers a needle-free delivery approach wherein a dry powder formulation of microscopic particles, such as polynucleotide or polypeptide particles, are accelerated to high speed within a helium gas jet generated by a hand held device, propelling the particles into a target tissue of interest.

In a related embodiment, other devices and methods that may be useful for gas-driven needle-less injection of compositions of the present invention include those provided by Bioject, Inc. (Portland, OR), some examples of which are described in U.S. Patent Nos. 4,790,824; 5,064,413; 5,312,335; 5,383,851; 5,399,163; 5,520,639 and 5,993,412.

According to another embodiment, the pharmaceutical compositions described herein will comprise one or more immunostimulants in addition to the immunogenic polynucleotide, polypeptide, antibody, T-cell, TCR, and/or APC compositions of this invention. An immunostimulant refers to essentially any substance that enhances or potentiates an immune response (antibody and/or cell-mediated) to an exogenous antigen. One preferred type of immunostimulant comprises an adjuvant. Many adjuvants contain a substance designed to protect the antigen from rapid catabolism, such as aluminum hydroxide or mineral oil, and a stimulator of immune responses, such as lipid A, *Bortadella pertussis* or *Mycobacterium tuberculosis* derived proteins. Certain adjuvants are commercially available as, for example, Freund's Incomplete Adjuvant and Complete Adjuvant (Difco Laboratories, Detroit, MI); Merck Adjuvant 65 (Merck and Company, Inc., Rahway, NJ); AS-2 (SmithKline Beecham, Philadelphia, PA); aluminum salts such as aluminum hydroxide gel (alum) or aluminum phosphate; salts of calcium, iron or zinc; an insoluble suspension of acylated tyrosine; acylated sugars; cationically or anionically derivatized polysaccharides; polyphosphazenes; biodegradable microspheres; monophosphoryl lipid A and quil A. Cytokines, such as GM-CSF, interleukin-2, -7, -12, and other like growth factors, may also be used as adjuvants.

Within certain embodiments of the invention, the adjuvant composition is preferably one that induces an immune response predominantly of the Th1 type. High levels of Th1-type cytokines (*e.g.,* IFN-γ, TNFα, IL-2 and IL-12) tend to favor the induction of cell mediated immune responses to an administered antigen. In contrast, high levels of Th2-type cytokines (*e.g.,* IL-4, IL-5, IL-6 and IL-10) tend to favor the induction of humoral immune responses. Following application of a vaccine as provided herein, a patient will support an immune response that includes Th1- and Th2-type responses. Within a preferred embodiment, in which a response is predominantly Th1-type, the level of Th1-type cytokines will increase to a greater extent than the level of Th2-type cytokines. The levels of these cytokines may be readily assessed using standard assays. For a review of the families of cytokines, see Mosmann and Coffman, *Ann. Rev. Immunol. 7*:145-173, 1989.

Certain preferred adjuvants for eliciting a predominantly Th1-type response include, for example, a combination of monophosphoryl lipid A, preferably 3-de-O-acylated monophosphoryl lipid A, together with an aluminum salt. MPL® adjuvants are available from Corixa Corporation (Seattle, WA; *see*, for example, US Patent Nos. 4,436,727; 4,877,611; 4,866,034 and 4,912,094). CpG-containing oligonucleotides (in which the CpG dinucleotide is unmethylated) also induce a predominantly Th1 response. Such oligonucleotides are well known and are described, for example, in WO 96/02555, WO 99/33488 and U.S. Patent Nos. 6,008,200 and 5,856,462. Immunostimulatory DNA sequences are also described, for example, by Sato et al., *Science 273*:352, 1996. Another preferred adjuvant comprises a saponin, such as Quil A, or derivatives thereof, including QS21 and QS7 (Aquila Biopharmaceuticals Inc., Framingham, MA); Escin; Digitonin; or *Gypsophila* or *Chenopodium quinoa* saponins . Other preferred formulations include more than one saponin in the adjuvant combinations of the present invention, for example combinations of at least two of the following group comprising QS21, QS7, Quil A, β-escin, or digitonin.

Alternatively the saponin formulations may be combined with vaccine vehicles composed of chitosan or other polycationic polymers, polylactide and polylactide-co-glycolide particles, poly-N-acetyl glucosamine-based polymer matrix,

particles composed of polysaccharides or chemically modified polysaccharides, liposomes and lipid-based particles, particles composed of glycerol monoesters, etc. The saponins may also be formulated in the presence of cholesterol to form particulate structures such as liposomes or ISCOMs. Furthermore, the saponins may be formulated together with a polyoxyethylene ether or ester, in either a non-particulate solution or suspension, or in a particulate structure such as a paucilamelar liposome or ISCOM. The saponins may also be formulated with excipients such as Carbopol^{R} to increase viscosity, or may be formulated in a dry powder form with a powder excipient such as lactose.

In one preferred embodiment, the adjuvant system includes the combination of a monophosphoryl lipid A and a saponin derivative, such as the combination of QS21 and 3D-MPL® adjuvant, as described in WO 94/00153, or a less reactogenic composition where the QS21 is quenched with cholesterol, as described in WO 96/33739. Other preferred formulations comprise an oil-in-water emulsion and tocopherol. Another particularly preferred adjuvant formulation employing QS21, 3D-MPL® adjuvant and tocopherol in an oil-in-water emulsion is described in WO 95/17210.

Another enhanced adjuvant system involves the combination of a CpG-containing oligonucleotide and a saponin derivative particularly the combination of CpG and QS21 is disclosed in WO 00/09159. Preferably the formulation additionally comprises an oil in water emulsion and tocopherol.

Additional illustrative adjuvants for use in the pharmaceutical compositions of the invention include Montanide ISA 720 (Seppic, France), SAF (Chiron, California, United States), ISCOMS (CSL), MF-59 (Chiron), the SBAS series of adjuvants (*e.g.*, SBAS-2 or SBAS-4, available from SmithKline Beecham, Rixensart, Belgium), Detox (Enhanzyn®) (Corixa, Hamilton, MT), RC-529 (Corixa, Hamilton, MT) and other aminoalkyl glucosaminide 4-phosphates (AGPs), such as those described in pending U.S. Patent Application Serial Nos. 08/853,826 and 09/074,720, the disclosures of which are incorporated herein by reference in their entireties, and polyoxyethylene ether adjuvants such as those described in WO 99/52549A1.

Other preferred adjuvants include adjuvant molecules of the general formula

(I): $HO(CH_2CH_2O)_n$-A-R,

wherein, n is 1-50, A is a bond or –C(O)-, R is C_{1-50} alkyl or Phenyl C_{1-50} alkyl.

One embodiment of the present invention consists of a vaccine formulation comprising a polyoxyethylene ether of general formula (I), wherein n is between 1 and 50, preferably 4-24, most preferably 9; the R component is C_{1-50}, preferably C_4-C_{20} alkyl and most preferably C_{12} alkyl, and A is a bond. The concentration of the polyoxyethylene ethers should be in the range 0.1-20%, preferably from 0.1-10%, and most preferably in the range 0.1-1%. Preferred polyoxyethylene ethers are selected from the following group: polyoxyethylene-9-lauryl ether, polyoxyethylene-9-steoryl ether, polyoxyethylene-8-steoryl ether, polyoxyethylene-4-lauryl ether, polyoxyethylene-35-lauryl ether, and polyoxyethylene-23-lauryl ether. Polyoxyethylene ethers such as polyoxyethylene lauryl ether are described in the Merck index (12^{th} edition: entry 7717). These adjuvant molecules are described in WO 99/52549.

The polyoxyethylene ether according to the general formula (I) above may, if desired, be combined with another adjuvant. For example, a preferred adjuvant combination is preferably with CpG as described in the pending UK patent application GB 9820956.2.

According to another embodiment of this invention, an immunogenic composition described herein is delivered to a host via antigen presenting cells (APCs), such as dendritic cells, macrophages, B cells, monocytes and other cells that may be engineered to be efficient APCs. Such cells may, but need not, be genetically modified to increase the capacity for presenting the antigen, to improve activation and/or maintenance of the T cell response, to have anti-tumor effects *per se* and/or to be immunologically compatible with the receiver (*i.e.,* matched HLA haplotype). APCs may generally be isolated from any of a variety of biological fluids and organs, including tumor and peritumoral tissues, and may be autologous, allogeneic, syngeneic or xenogeneic cells.

Certain preferred embodiments of the present invention use dendritic cells or progenitors thereof as antigen-presenting cells. Dendritic cells are highly potent APCs (Banchereau and Steinman, *Nature 392*:245-251, 1998) and have been shown to be effective as a physiological adjuvant for eliciting prophylactic or therapeutic antitumor immunity (*see* Timmerman and Levy, *Ann. Rev. Med. 50*:507-529, 1999). In general, dendritic cells may be identified based on their typical shape (stellate *in situ*, with marked cytoplasmic processes (dendrites) visible *in vitro*), their ability to take up, process and present antigens with high efficiency and their ability to activate naïve T cell responses. Dendritic cells may, of course, be engineered to express specific cell-surface receptors or ligands that are not commonly found on dendritic cells *in vivo* or *ex vivo*, and such modified dendritic cells are contemplated by the present invention. As an alternative to dendritic cells, secreted vesicles antigen-loaded dendritic cells (called exosomes) may be used within a vaccine (*see* Zitvogel et al., *Nature Med. 4*:594-600, 1998).

Dendritic cells and progenitors may be obtained from peripheral blood, bone marrow, tumor-infiltrating cells, peritumoral tissues-infiltrating cells, lymph nodes, spleen, skin, umbilical cord blood or any other suitable tissue or fluid. For example, dendritic cells may be differentiated *ex vivo* by adding a combination of cytokines such as GM-CSF, IL-4, IL-13 and/or TNFα to cultures of monocytes harvested from peripheral blood. Alternatively, CD34 positive cells harvested from peripheral blood, umbilical cord blood or bone marrow may be differentiated into dendritic cells by adding to the culture medium combinations of GM-CSF, IL-3, TNFα, CD40 ligand, LPS, flt3 ligand and/or other compound(s) that induce differentiation, maturation and proliferation of dendritic cells.

Dendritic cells are conveniently categorized as "immature" and "mature" cells, which allows a simple way to discriminate between two well characterized phenotypes. However, this nomenclature should not be construed to exclude all possible intermediate stages of differentiation. Immature dendritic cells are characterized as APC with a high capacity for antigen uptake and processing, which correlates with the high expression of Fcγ receptor and mannose receptor. The mature phenotype is typically characterized by a lower expression of these markers, but a high

expression of cell surface molecules responsible for T cell activation such as class I and class II MHC, adhesion molecules (*e.g.*, CD54 and CD11) and costimulatory molecules (*e.g.*, CD40, CD80, CD86 and 4-1BB).

APCs may generally be transfected with a polynucleotide of the invention (or portion or other variant thereof) such that the encoded polypeptide, or an immunogenic portion thereof, is expressed on the cell surface. Such transfection may take place *ex vivo*, and a pharmaceutical composition comprising such transfected cells may then be used for therapeutic purposes, as described herein. Alternatively, a gene delivery vehicle that targets a dendritic or other antigen presenting cell may be administered to a patient, resulting in transfection that occurs *in vivo*. *In vivo* and *ex vivo* transfection of dendritic cells, for example, may generally be performed using any methods known in the art, such as those described in WO 97/24447, or the gene gun approach described by Mahvi et al., *Immunology and cell Biology 75*:456-460, 1997. Antigen loading of dendritic cells may be achieved by incubating dendritic cells or progenitor cells with the tumor polypeptide, DNA (naked or within a plasmid vector) or RNA; or with antigen-expressing recombinant bacterium or viruses (*e.g.*, vaccinia, fowlpox, adenovirus or lentivirus vectors). Prior to loading, the polypeptide may be covalently conjugated to an immunological partner that provides T cell help (*e.g.*, a carrier molecule). Alternatively, a dendritic cell may be pulsed with a non-conjugated immunological partner, separately or in the presence of the polypeptide.

While any suitable carrier known to those of ordinary skill in the art may be employed in the pharmaceutical compositions of this invention, the type of carrier will typically vary depending on the mode of administration. Compositions of the present invention may be formulated for any appropriate manner of administration, including for example, topical, oral, nasal, mucosal, intravenous, intracranial, intraperitoneal, subcutaneous and intramuscular administration.

Carriers for use within such pharmaceutical compositions are biocompatible, and may also be biodegradable. In certain embodiments, the formulation preferably provides a relatively constant level of active component release. In other embodiments, however, a more rapid rate of release immediately upon administration may be desired. The formulation of such compositions is well within the

level of ordinary skill in the art using known techniques. Illustrative carriers useful in this regard include microparticles of poly(lactide-co-glycolide), polyacrylate, latex, starch, cellulose, dextran and the like. Other illustrative delayed-release carriers include supramolecular biovectors, which comprise a non-liquid hydrophilic core (*e.g.*, a cross-linked polysaccharide or oligosaccharide) and, optionally, an external layer comprising an amphiphilic compound, such as a phospholipid (*see e.g.*, U.S. Patent No. 5,151,254 and PCT applications WO 94/20078, WO/94/23701 and WO 96/06638). The amount of active compound contained within a sustained release formulation depends upon the site of implantation, the rate and expected duration of release and the nature of the condition to be treated or prevented.

In another illustrative embodiment, biodegradable microspheres (*e.g.*, polylactate polyglycolate) are employed as carriers for the compositions of this invention. Suitable biodegradable microspheres are disclosed, for example, in U.S. Patent Nos. 4,897,268; 5,075,109; 5,928,647; 5,811,128; 5,820,883; 5,853,763; 5,814,344, 5,407,609 and 5,942,252. Modified hepatitis B core protein carrier systems. such as described in WO/99 40934, and references cited therein, will also be useful for many applications. Another illustrative carrier/delivery system employs a carrier comprising particulate-protein complexes, such as those described in U.S. Patent No. 5,928,647, which are capable of inducing a class I-restricted cytotoxic T lymphocyte responses in a host.

In another illustrative embodiment, calcium phosphate core particles are employed as carriers, vaccine adjuvants, or as controlled release matrices for the compositions of this invention. Exemplary calcium phosphate particles are disclosed, for example, in published patent application No. WO/0046147.

The pharmaceutical compositions of the invention will often further comprise one or more buffers (*e.g.*, neutral buffered saline or phosphate buffered saline), carbohydrates (*e.g.*, glucose, mannose, sucrose or dextrans), mannitol, proteins, polypeptides or amino acids such as glycine, antioxidants, bacteriostats, chelating agents such as EDTA or glutathione, adjuvants (*e.g.*, aluminum hydroxide), solutes that render the formulation isotonic, hypotonic or weakly hypertonic with the blood of a

recipient, suspending agents, thickening agents and/or preservatives. Alternatively, compositions of the present invention may be formulated as a lyophilizate.

The pharmaceutical compositions described herein may be presented in unit-dose or multi-dose containers, such as sealed ampoules or vials. Such containers are typically sealed in such a way to preserve the sterility and stability of the formulation until use. In general, formulations may be stored as suspensions, solutions or emulsions in oily or aqueous vehicles. Alternatively, a pharmaceutical composition may be stored in a freeze-dried condition requiring only the addition of a sterile liquid carrier immediately prior to use.

The development of suitable dosing and treatment regimens for using the particular compositions described herein in a variety of treatment regimens, including *e.g.,* oral, parenteral, intravenous, intranasal, and intramuscular administration and formulation, is well known in the art, some of which are briefly discussed below for general purposes of illustration.

In certain applications, the pharmaceutical compositions disclosed herein may be delivered *via* oral administration to an animal. As such, these compositions may be formulated with an inert diluent or with an assimilable edible carrier, or they may be enclosed in hard- or soft-shell gelatin capsule, or they may be compressed into tablets, or they may be incorporated directly with the food of the diet.

The active compounds may even be incorporated with excipients and used in the form of ingestible tablets, buccal tables, troches, capsules, elixirs, suspensions, syrups, wafers, and the like (see, for example, Mathiowitz *et al.*, Nature 1997 Mar 27;386(6623):410-4; Hwang *et al.*, Crit Rev Ther Drug Carrier Syst 1998;15(3):243-84; U. S. Patent 5,641,515; U. S. Patent 5,580,579 and U. S. Patent 5,792,451). Tablets, troches, pills, capsules and the like may also contain any of a variety of additional components, for example, a binder, such as gum tragacanth, acacia, cornstarch, or gelatin; excipients, such as dicalcium phosphate; a disintegrating agent, such as corn starch, potato starch, alginic acid and the like; a lubricant, such as magnesium stearate; and a sweetening agent, such as sucrose, lactose or saccharin may be added or a flavoring agent, such as peppermint, oil of wintergreen, or cherry flavoring. When the dosage unit form is a capsule, it may contain, in addition to

materials of the above type, a liquid carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules may be coated with shellac, sugar, or both. Of course, any material used in preparing any dosage unit form should be pharmaceutically pure and substantially non-toxic in the amounts employed. In addition, the active compounds may be incorporated into sustained-release preparation and formulations.

Typically, these formulations will contain at least about 0.1% of the active compound or more, although the percentage of the active ingredient(s) may, of course, be varied and may conveniently be between about 1 or 2% and about 60% or 70% or more of the weight or volume of the total formulation. Naturally, the amount of active compound(s) in each therapeutically useful composition may be prepared is such a way that a suitable dosage will be obtained in any given unit dose of the compound. Factors such as solubility, bioavailability, biological half-life, route of administration, product shelf life, as well as other pharmacological considerations will be contemplated by one skilled in the art of preparing such pharmaceutical formulations, and as such, a variety of dosages and treatment regimens may be desirable.

For oral administration the compositions of the present invention may alternatively be incorporated with one or more excipients in the form of a mouthwash, dentifrice, buccal tablet, oral spray, or sublingual orally-administered formulation. Alternatively, the active ingredient may be incorporated into an oral solution such as one containing sodium borate, glycerin and potassium bicarbonate, or dispersed in a dentifrice, or added in a therapeutically-effective amount to a composition that may include water, binders, abrasives, flavoring agents, foaming agents, and humectants. Alternatively the compositions may be fashioned into a tablet or solution form that may be placed under the tongue or otherwise dissolved in the mouth.

In certain circumstances it will be desirable to deliver the pharmaceutical compositions disclosed herein parenterally, intravenously, intramuscularly, or even intraperitoneally. Such approaches are well known to the skilled artisan, some of which are further described, for example, in U. S. Patent 5,543,158; U. S. Patent 5,641,515 and U. S. Patent 5,399,363. In certain embodiments, solutions of the active compounds as free base or pharmacologically acceptable salts may be prepared in water suitably

mixed with a surfactant, such as hydroxypropylcellulose. Dispersions may also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations generally will contain a preservative to prevent the growth of microorganisms.

Illustrative pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions (for example, see U. S. Patent 5,466,468). In all cases the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (*e.g.,* glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and/or vegetable oils. Proper fluidity may be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and/or by the use of surfactants. The prevention of the action of microorganisms can be facilitated by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

In one embodiment, for parenteral administration in an aqueous solution, the solution should be suitably buffered if necessary and the liquid diluent first rendered isotonic with sufficient saline or glucose. These particular aqueous solutions are especially suitable for intravenous, intramuscular, subcutaneous and intraperitoneal administration. In this connection, a sterile aqueous medium that can be employed will be known to those of skill in the art in light of the present disclosure. For example, one dosage may be dissolved in 1 ml of isotonic NaCl solution and either added to 1000 ml of hypodermoclysis fluid or injected at the proposed site of infusion, (see for example, "Remington's Pharmaceutical Sciences" 15th Edition, pages 1035-1038 and 1570-

1580). Some variation in dosage will necessarily occur depending on the condition of the subject being treated. Moreover, for human administration, preparations will of course preferably meet sterility, pyrogenicity, and the general safety and purity standards as required by FDA Office of Biologics standards.

In another embodiment of the invention, the compositions disclosed herein may be formulated in a neutral or salt form. Illustrative pharmaceutically-acceptable salts include the acid addition salts (formed with the free amino groups of the protein) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, histidine, procaine and the like. Upon formulation, solutions will be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically effective.

The carriers can further comprise any and all solvents, dispersion media, vehicles, coatings, diluents, antibacterial and antifungal agents, isotonic and absorption delaying agents, buffers, carrier solutions, suspensions, colloids, and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, its use in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions. The phrase "pharmaceutically-acceptable" refers to molecular entities and compositions that do not produce an allergic or similar untoward reaction when administered to a human.

In certain embodiments, the pharmaceutical compositions may be delivered by intranasal sprays, inhalation, and/or other aerosol delivery vehicles. Methods for delivering genes, nucleic acids, and peptide compositions directly to the lungs *via* nasal aerosol sprays has been described, *e.g.*, in U. S. Patent 5,756,353 and U. S. Patent 5,804,212. Likewise, the delivery of drugs using intranasal microparticle resins (Takenaga *et al.*, J Controlled Release 1998 Mar 2;52(1-2):81-7) and lysophosphatidyl-glycerol compounds (U. S. Patent 5,725,871) are also well-known in

the pharmaceutical arts. Likewise, illustrative transmucosal drug delivery in the form of a polytetrafluoroetheylene support matrix is described in U. S. Patent 5,780,045.

In certain embodiments, liposomes, nanocapsules, microparticles, lipid particles, vesicles, and the like, are used for the introduction of the compositions of the present invention into suitable host cells/organisms. In particular, the compositions of the present invention may be formulated for delivery either encapsulated in a lipid particle, a liposome, a vesicle, a nanosphere, or a nanoparticle or the like. Alternatively, compositions of the present invention can be bound, either covalently or non-covalently, to the surface of such carrier vehicles.

The formation and use of liposome and liposome-like preparations as potential drug carriers is generally known to those of skill in the art (see for example, Lasic, Trends Biotechnol 1998 Jul;16(7):307-21; Takakura, Nippon Rinsho 1998 Mar;56(3):691-5; Chandran *et al.*, Indian J Exp Biol. 1997 Aug;35(8):801-9; Margalit, Crit Rev Ther Drug Carrier Syst. 1995;12(2-3):233-61; U.S. Patent 5,567,434; U.S. Patent 5,552,157; U.S. Patent 5,565,213; U.S. Patent 5,738,868 and U.S. Patent 5,795,587, each specifically incorporated herein by reference in its entirety).

Liposomes have been used successfully with a number of cell types that are normally difficult to transfect by other procedures, including T cell suspensions, primary hepatocyte cultures and PC 12 cells (Renneisen *et al.*, J Biol Chem. 1990 Sep 25;265(27):16337-42; Muller *et al.*, DNA Cell Biol. 1990 Apr;9(3):221-9). In addition, liposomes are free of the DNA length constraints that are typical of viral-based delivery systems. Liposomes have been used effectively to introduce genes, various drugs, radiotherapeutic agents, enzymes, viruses, transcription factors, allosteric effectors and the like, into a variety of cultured cell lines and animals. Furthermore, he use of liposomes does not appear to be associated with autoimmune responses or unacceptable toxicity after systemic delivery.

In certain embodiments, liposomes are formed from phospholipids that are dispersed in an aqueous medium and spontaneously form multilamellar concentric bilayer vesicles (also termed multilamellar vesicles (MLVs).

Alternatively, in other embodiments, the invention provides for pharmaceutically-acceptable nanocapsule formulations of the compositions of the

present invention. Nanocapsules can generally entrap compounds in a stable and reproducible way (see, for example, Quintanar-Guerrero *et al.*, Drug Dev Ind Pharm. 1998 Dec;24(12):1113-28). To avoid side effects due to intracellular polymeric overloading, such ultrafine particles (sized around 0.1 μm) may be designed using polymers able to be degraded *in vivo*. Such particles can be made as described, for example, by Couvreur *et al.*, Crit Rev Ther Drug Carrier Syst. 1988;5(1):1-20; zur Muhlen *et al.*, Eur J Pharm Biopharm. 1998 Mar;45(2):149-55; Zambaux *et al.* J Controlled Release. 1998 Jan 2;50(1-3):31-40; and U. S. Patent 5,145,684.

CANCER THERAPEUTIC METHODS

Immunologic approaches to cancer therapy are based on the recognition that cancer cells can often evade the body's defenses against aberrant or foreign cells and molecules, and that these defenses might be therapeutically stimulated to regain the lost ground, *e.g.* pgs. 623-648 in Klein, Immunology (Wiley-Interscience, New York, 1982). Numerous recent observations that various immune effectors can directly or indirectly inhibit growth of tumors has led to renewed interest in this approach to cancer therapy, *e.g.* Jager, et al., Oncology 2001;60(1):1-7; Renner, et al., Ann Hematol 2000 Dec;79(12):651-9.

Four-basic cell types whose function has been associated with antitumor cell immunity and the elimination of tumor cells from the body are: i) B-lymphocytes which secrete immunoglobulins into the blood plasma for identifying and labeling the nonself invader cells; ii) monocytes which secrete the complement proteins that are responsible for lysing and processing the immunoglobulin-coated target invader cells; iii) natural killer lymphocytes having two mechanisms for the destruction of tumor cells, antibody-dependent cellular cytotoxicity and natural killing; and iv) T-lymphocytes possessing antigen-specific receptors and having the capacity to recognize a tumor cell carrying complementary marker molecules (Schreiber, H., 1989, in Fundamental Immunology (ed). W. E. Paul, pp. 923-955).

Cancer immunotherapy generally focuses on inducing humoral immune responses, cellular immune responses, or both. Moreover, it is well established that induction of $CD4^+$ T helper cells is necessary in order to secondarily induce either

antibodies or cytotoxic $CD8^+$ T cells. Polypeptide antigens that are selective or ideally specific for cancer cells, particularly colon cancer cells, offer a powerful approach for inducing immune responses against colon cancer, and are an important aspect of the present invention.

Therefore, in further aspects of the present invention, the pharmaceutical compositions described herein may be used to stimulate an immune response against cancer, particularly for the immunotherapy of colon cancer. Within such methods, the pharmaceutical compositions described herein are administered to a patient, typically a warm-blooded animal, preferably a human. A patient may or may not be afflicted with cancer. Pharmaceutical compositions and vaccines may be administered either prior to or following surgical removal of primary tumors and/or treatment such as administration of radiotherapy or conventional chemotherapeutic drugs. As discussed above, administration of the pharmaceutical compositions may be by any suitable method, including administration by intravenous, intraperitoneal, intramuscular, subcutaneous, intranasal, intradermal, anal, vaginal, topical and oral routes.

Within certain embodiments, immunotherapy may be active immunotherapy, in which treatment relies on the *in vivo* stimulation of the endogenous host immune system to react against tumors with the administration of immune response-modifying agents (such as polypeptides and polynucleotides as provided herein).

Within other embodiments, immunotherapy may be passive immunotherapy, in which treatment involves the delivery of agents with established tumor-immune reactivity (such as effector cells or antibodies) that can directly or indirectly mediate antitumor effects and does not necessarily depend on an intact host immune system. Examples of effector cells include T cells as discussed above, T lymphocytes (such as $CD8^+$ cytotoxic T lymphocytes and $CD4^+$ T-helper tumor-infiltrating lymphocytes), killer cells (such as Natural Killer cells and lymphokine-activated killer cells), B cells and antigen-presenting cells (such as dendritic cells and macrophages) expressing a polypeptide provided herein. T cell receptors and antibody receptors specific for the polypeptides recited herein may be cloned, expressed and transferred into other vectors or effector cells for adoptive immunotherapy. The

polypeptides provided herein may also be used to generate antibodies or anti-idiotypic antibodies (as described above and in U.S. Patent No. 4,918,164) for passive immunotherapy.

Monoclonal antibodies may be labeled with any of a variety of labels for desired selective usages in detection, diagnostic assays or therapeutic applications (as described in U.S. Patent Nos. 6,090,365; 6,015,542; 5,843,398; 5,595,721; and 4,708,930, hereby incorporated by reference in their entirety as if each was incorporated individually). In each case, the binding of the labelled monoclonal antibody to the determinant site of the antigen will signal detection or delivery of a particular therapeutic agent to the antigenic determinant on the non-normal cell. A further object of this invention is to provide the specific monoclonal antibody suitably labelled for achieving such desired selective usages thereof.

Effector cells may generally be obtained in sufficient quantities for adoptive immunotherapy by growth *in vitro*, as described herein. Culture conditions for expanding single antigen-specific effector cells to several billion in number with retention of antigen recognition *in vivo* are well known in the art. Such *in vitro* culture conditions typically use intermittent stimulation with antigen, often in the presence of cytokines (such as IL-2) and non-dividing feeder cells. As noted above, immunoreactive polypeptides as provided herein may be used to rapidly expand antigen-specific T cell cultures in order to generate a sufficient number of cells for immunotherapy. In particular, antigen-presenting cells, such as dendritic, macrophage, monocyte, fibroblast and/or B cells, may be pulsed with immunoreactive polypeptides or transfected with one or more polynucleotides using standard techniques well known in the art. For example, antigen-presenting cells can be transfected with a polynucleotide having a promoter appropriate for increasing expression in a recombinant virus or other expression system. Cultured effector cells for use in therapy must be able to grow and distribute widely, and to survive long term *in vivo*. Studies have shown that cultured effector cells can be induced to grow in vivo and to survive long term in substantial numbers by repeated stimulation with antigen supplemented with IL-2 (*see*, for example, Cheever et al., *Immunological Reviews 157*:177, 1997).

Alternatively, a vector expressing a polypeptide recited herein may be introduced into antigen presenting cells taken from a patient and clonally propagated *ex vivo* for transplant back into the same patient. Transfected cells may be reintroduced into the patient using any means known in the art, preferably in sterile form by intravenous, intracavitary, intraperitoneal or intratumor administration.

Routes and frequency of administration of the therapeutic compositions described herein, as well as dosage, will vary from individual to individual, and may be readily established using standard techniques. In general, the pharmaceutical compositions and vaccines may be administered by injection (*e.g.*, intracutaneous, intramuscular, intravenous or subcutaneous), intranasally (*e.g.*, by aspiration) or orally. Preferably, between 1 and 10 doses may be administered over a 52 week period. Preferably, 6 doses are administered, at intervals of 1 month, and booster vaccinations may be given periodically thereafter. Alternate protocols may be appropriate for individual patients. A suitable dose is an amount of a compound that, when administered as described above, is capable of promoting an anti-tumor immune response, and is at least 10-50% above the basal (*i.e.*, untreated) level. Such response can be monitored by measuring the anti-tumor antibodies in a patient or by vaccine-dependent generation of cytolytic effector cells capable of killing the patient's tumor cells *in vitro*. Such vaccines should also be capable of causing an immune response that leads to an improved clinical outcome (*e.g.*, more frequent remissions, complete or partial or longer disease-free survival) in vaccinated patients as compared to non-vaccinated patients. In general, for pharmaceutical compositions and vaccines comprising one or more polypeptides, the amount of each polypeptide present in a dose ranges from about 25 μg to 5 mg per kg of host. Suitable dose sizes will vary with the size of the patient, but will typically range from about 0.1 mL to about 5 mL.

In general, an appropriate dosage and treatment regimen provides the active compound(s) in an amount sufficient to provide therapeutic and/or prophylactic benefit. Such a response can be monitored by establishing an improved clinical outcome (*e.g.*, more frequent remissions, complete or partial, or longer disease-free survival) in treated patients as compared to non-treated patients. Increases in preexisting immune responses to a tumor protein generally correlate with an improved

clinical outcome. Such immune responses may generally be evaluated using standard proliferation, cytotoxicity or cytokine assays, which may be performed using samples obtained from a patient before and after treatment.

CANCER DETECTION AND DIAGNOSTIC COMPOSITIONS, METHODS AND KITS

In general, a cancer may be detected in a patient based on the presence of one or more colon tumor proteins and/or polynucleotides encoding such proteins in a biological sample (for example, blood, sera, sputum urine and/or tumor biopsies) obtained from the patient. In other words, such proteins may be used as markers to indicate the presence or absence of a cancer such as colon cancer. In addition, such proteins may be useful for the detection of other cancers. The binding agents provided herein generally permit detection of the level of antigen that binds to the agent in the biological sample.

Polynucleotide primers and probes may be used to detect the level of mRNA encoding a tumor protein, which is also indicative of the presence or absence of a cancer. In general, a tumor sequence should be present at a level that is at least two-fold, preferably three-fold, and more preferably five-fold or higher in tumor tissue than in normal tissue of the same type from which the tumor arose. Expression levels of a particular tumor sequence in tissue types different from that in which the tumor arose are irrelevant in certain diagnostic embodiments since the presence of tumor cells can be confirmed by observation of predetermined differential expression levels, e.g., 2-fold, 5-fold, etc, in tumor tissue to expression levels in normal tissue of the same type.

Other differential expression patterns can be utilized advantageously for diagnostic purposes. For example, in one aspect of the invention, overexpression of a tumor sequence in tumor tissue and normal tissue of the same type, but not in other normal tissue types, *e.g.* PBMCs, can be exploited diagnostically. In this case, the presence of metastatic tumor cells, for example in a sample taken from the circulation or some other tissue site different from that in which the tumor arose, can be identified and/or confirmed by detecting expression of the tumor sequence in the sample, for example using RT-PCR analysis. In many instances, it will be desired to enrich for

tumor cells in the sample of interest, e.g., PBMCs, using cell capture or other like techniques.

There are a variety of assay formats known to those of ordinary skill in the art for using a binding agent to detect polypeptide markers in a sample. *See, e.g.*, Harlow and Lane, *Antibodies: A Laboratory Manual,* Cold Spring Harbor Laboratory, 1988. In general, the presence or absence of a cancer in a patient may be determined by (a) contacting a biological sample obtained from a patient with a binding agent; (b) detecting in the sample a level of polypeptide that binds to the binding agent; and (c) comparing the level of polypeptide with a predetermined cut-off value.

In a preferred embodiment, the assay involves the use of binding agent immobilized on a solid support to bind to and remove the polypeptide from the remainder of the sample. The bound polypeptide may then be detected using a detection reagent that contains a reporter group and specifically binds to the binding agent/polypeptide complex. Such detection reagents may comprise, for example, a binding agent that specifically binds to the polypeptide or an antibody or other agent that specifically binds to the binding agent, such as an anti-immunoglobulin, protein G, protein A or a lectin. Alternatively, a competitive assay may be utilized, in which a polypeptide is labeled with a reporter group and allowed to bind to the immobilized binding agent after incubation of the binding agent with the sample. The extent to which components of the sample inhibit the binding of the labeled polypeptide to the binding agent is indicative of the reactivity of the sample with the immobilized binding agent. Suitable polypeptides for use within such assays include full length colon tumor proteins and polypeptide portions thereof to which the binding agent binds, as described above.

The solid support may be any material known to those of ordinary skill in the art to which the tumor protein may be attached. For example, the solid support may be a test well in a microtiter plate or a nitrocellulose or other suitable membrane. Alternatively, the support may be a bead or disc, such as glass, fiberglass, latex or a plastic material such as polystyrene or polyvinylchloride. The support may also be a magnetic particle or a fiber optic sensor, such as those disclosed, for example, in U.S. Patent No. 5,359,681. The binding agent may be immobilized on the solid support

using a variety of techniques known to those of skill in the art, which are amply described in the patent and scientific literature. In the context of the present invention, the term "immobilization" refers to both noncovalent association, such as adsorption, and covalent attachment (which may be a direct linkage between the agent and functional groups on the support or may be a linkage by way of a cross-linking agent). Immobilization by adsorption to a well in a microtiter plate or to a membrane is preferred. In such cases, adsorption may be achieved by contacting the binding agent, in a suitable buffer, with the solid support for a suitable amount of time. The contact time varies with temperature, but is typically between about 1 hour and about 1 day. In general, contacting a well of a plastic microtiter plate (such as polystyrene or polyvinylchloride) with an amount of binding agent ranging from about 10 ng to about 10 μg, and preferably about 100 ng to about 1 μg, is sufficient to immobilize an adequate amount of binding agent.

Covalent attachment of binding agent to a solid support may generally be achieved by first reacting the support with a bifunctional reagent that will react with both the support and a functional group, such as a hydroxyl or amino group, on the binding agent. For example, the binding agent may be covalently attached to supports having an appropriate polymer coating using benzoquinone or by condensation of an aldehyde group on the support with an amine and an active hydrogen on the binding partner (*see*, *e.g.*, Pierce Immunotechnology Catalog and Handbook, 1991, at A12-A13).

In certain embodiments, the assay is a two-antibody sandwich assay. This assay may be performed by first contacting an antibody that has been immobilized on a solid support, commonly the well of a microtiter plate, with the sample, such that polypeptides within the sample are allowed to bind to the immobilized antibody. Unbound sample is then removed from the immobilized polypeptide-antibody complexes and a detection reagent (preferably a second antibody capable of binding to a different site on the polypeptide) containing a reporter group is added. The amount of detection reagent that remains bound to the solid support is then determined using a method appropriate for the specific reporter group.

More specifically, once the antibody is immobilized on the support as described above, the remaining protein binding sites on the support are typically blocked. Any suitable blocking agent known to those of ordinary skill in the art, such as bovine serum albumin or Tween 20™ (Sigma Chemical Co., St. Louis, MO). The immobilized antibody is then incubated with the sample, and polypeptide is allowed to bind to the antibody. The sample may be diluted with a suitable diluent, such as phosphate-buffered saline (PBS) prior to incubation. In general, an appropriate contact time (*i.e.*, incubation time) is a period of time that is sufficient to detect the presence of polypeptide within a sample obtained from an individual with colon cancer at least about 95% of that achieved at equilibrium between bound and unbound polypeptide. Those of ordinary skill in the art will recognize that the time necessary to achieve equilibrium may be readily determined by assaying the level of binding that occurs over a period of time. At room temperature, an incubation time of about 30 minutes is generally sufficient.

Unbound sample may then be removed by washing the solid support with an appropriate buffer, such as PBS containing 0.1% Tween 20™. The second antibody, which contains a reporter group, may then be added to the solid support. Preferred reporter groups include those groups recited above.

The detection reagent is then incubated with the immobilized antibody-polypeptide complex for an amount of time sufficient to detect the bound polypeptide. An appropriate amount of time may generally be determined by assaying the level of binding that occurs over a period of time. Unbound detection reagent is then removed and bound detection reagent is detected using the reporter group. The method employed for detecting the reporter group depends upon the nature of the reporter group. For radioactive groups, scintillation counting or autoradiographic methods are generally appropriate. Spectroscopic methods may be used to detect dyes, luminescent groups and fluorescent groups. Biotin may be detected using avidin, coupled to a different reporter group (commonly a radioactive or fluorescent group or an enzyme). Enzyme reporter groups may generally be detected by the addition of substrate (generally for a specific period of time), followed by spectroscopic or other analysis of the reaction products.

To determine the presence or absence of a cancer, such as colon cancer, the signal detected from the reporter group that remains bound to the solid support is generally compared to a signal that corresponds to a predetermined cut-off value. In one preferred embodiment, the cut-off value for the detection of a cancer is the average mean signal obtained when the immobilized antibody is incubated with samples from patients without the cancer. In general, a sample generating a signal that is three standard deviations above the predetermined cut-off value is considered positive for the cancer. In an alternate preferred embodiment, the cut-off value is determined using a Receiver Operator Curve, according to the method of Sackett et al., *Clinical Epidemiology: A Basic Science for Clinical Medicine*, Little Brown and Co., 1985, p. 106-7. Briefly, in this embodiment, the cut-off value may be determined from a plot of pairs of true positive rates (*i.e.*, sensitivity) and false positive rates (100%-specificity) that correspond to each possible cut-off value for the diagnostic test result. The cut-off value on the plot that is the closest to the upper left-hand corner (*i.e.*, the value that encloses the largest area) is the most accurate cut-off value, and a sample generating a signal that is higher than the cut-off value determined by this method may be considered positive. Alternatively, the cut-off value may be shifted to the left along the plot, to minimize the false positive rate, or to the right, to minimize the false negative rate. In general, a sample generating a signal that is higher than the cut-off value determined by this method is considered positive for a cancer.

In a related embodiment, the assay is performed in a flow-through or strip test format, wherein the binding agent is immobilized on a membrane, such as nitrocellulose. In the flow-through test, polypeptides within the sample bind to the immobilized binding agent as the sample passes through the membrane. A second, labeled binding agent then binds to the binding agent-polypeptide complex as a solution containing the second binding agent flows through the membrane. The detection of bound second binding agent may then be performed as described above. In the strip test format, one end of the membrane to which binding agent is bound is immersed in a solution containing the sample. The sample migrates along the membrane through a region containing second binding agent and to the area of immobilized binding agent. Concentration of second binding agent at the area of immobilized antibody indicates the

presence of a cancer. Typically, the concentration of second binding agent at that site generates a pattern, such as a line, that can be read visually. The absence of such a pattern indicates a negative result. In general, the amount of binding agent immobilized on the membrane is selected to generate a visually discernible pattern when the biological sample contains a level of polypeptide that would be sufficient to generate a positive signal in the two-antibody sandwich assay, in the format discussed above. Preferred binding agents for use in such assays are antibodies and antigen-binding fragments thereof. Preferably, the amount of antibody immobilized on the membrane ranges from about 25 ng to about 1 μg, and more preferably from about 50 ng to about 500 ng. Such tests can typically be performed with a very small amount of biological sample.

Of course, numerous other assay protocols exist that are suitable for use with the tumor proteins or binding agents of the present invention. The above descriptions are intended to be exemplary only. For example, it will be apparent to those of ordinary skill in the art that the above protocols may be readily modified to use tumor polypeptides to detect antibodies that bind to such polypeptides in a biological sample. The detection of such tumor protein specific antibodies may correlate with the presence of a cancer.

A cancer may also, or alternatively, be detected based on the presence of T cells that specifically react with a tumor protein in a biological sample. Within certain methods, a biological sample comprising $CD4^+$ and/or $CD8^+$ T cells isolated from a patient is incubated with a tumor polypeptide, a polynucleotide encoding such a polypeptide and/or an APC that expresses at least an immunogenic portion of such a polypeptide, and the presence or absence of specific activation of the T cells is detected. Suitable biological samples include, but are not limited to, isolated T cells. For example, T cells may be isolated from a patient by routine techniques (such as by Ficoll/Hypaque density gradient centrifugation of peripheral blood lymphocytes). T cells may be incubated *in vitro* for 2-9 days (typically 4 days) at 37°C with polypeptide (*e.g.*, 5 - 25 μg/ml). It may be desirable to incubate another aliquot of a T cell sample in the absence of tumor polypeptide to serve as a control. For $CD4^+$ T cells, activation is preferably detected by evaluating proliferation of the T cells. For $CD8^+$ T cells,

activation is preferably detected by evaluating cytolytic activity. A level of proliferation that is at least two fold greater and/or a level of cytolytic activity that is at least 20% greater than in disease-free patients indicates the presence of a cancer in the patient.

As noted above, a cancer may also, or alternatively, be detected based on the level of mRNA encoding a tumor protein in a biological sample. For example, at least two oligonucleotide primers may be employed in a polymerase chain reaction (PCR) based assay to amplify a portion of a tumor cDNA derived from a biological sample, wherein at least one of the oligonucleotide primers is specific for (*i.e.*, hybridizes to) a polynucleotide encoding the tumor protein. The amplified cDNA is then separated and detected using techniques well known in the art, such as gel electrophoresis.

Similarly, oligonucleotide probes that specifically hybridize to a polynucleotide encoding a tumor protein may be used in a hybridization assay to detect the presence of polynucleotide encoding the tumor protein in a biological sample.

To permit hybridization under assay conditions, oligonucleotide primers and probes should comprise an oligonucleotide sequence that has at least about 60%, preferably at least about 75% and more preferably at least about 90%, identity to a portion of a polynucleotide encoding a tumor protein of the invention that is at least 10 nucleotides, and preferably at least 20 nucleotides, in length. Preferably, oligonucleotide primers and/or probes hybridize to a polynucleotide encoding a polypeptide described herein under moderately stringent conditions, as defined above. Oligonucleotide primers and/or probes which may be usefully employed in the diagnostic methods described herein preferably are at least 10-40 nucleotides in length. In a preferred embodiment, the oligonucleotide primers comprise at least 10 contiguous nucleotides, more preferably at least 15 contiguous nucleotides, of a DNA molecule having a sequence as disclosed herein. Techniques for both PCR based assays and hybridization assays are well known in the art (*see,* for example, Mullis et al., *Cold Spring Harbor Symp. Quant. Biol.*, *51*:263, 1987; Erlich ed., *PCR Technology*, Stockton Press, NY, 1989).

One preferred assay employs RT-PCR, in which PCR is applied in conjunction with reverse transcription. Typically, RNA is extracted from a biological sample, such as biopsy tissue, and is reverse transcribed to produce cDNA molecules. PCR amplification using at least one specific primer generates a cDNA molecule, which may be separated and visualized using, for example, gel electrophoresis. Amplification may be performed on biological samples taken from a test patient and from an individual who is not afflicted with a cancer. The amplification reaction may be performed on several dilutions of cDNA spanning two orders of magnitude. A two-fold or greater increase in expression in several dilutions of the test patient sample as compared to the same dilutions of the non-cancerous sample is typically considered positive.

In another aspect of the present invention, cell capture technologies may be used in conjunction, with, for example, real-time PCR to provide a more sensitive tool for detection of metastatic cells expressing colon tumor antigens. Detection of colon cancer cells in biological samples, e.g., bone marrow samples, peripheral blood, and small needle aspiration samples is desirable for diagnosis and prognosis in colon cancer patients.

Immunomagnetic beads coated with specific monoclonal antibodies to surface cell markers, or tetrameric antibody complexes, may be used to first enrich or positively select cancer cells in a sample. Various commercially available kits may be used, including Dynabeads® Epithelial Enrich (Dynal Biotech, Oslo, Norway), StemSep™ (StemCell Technologies, Inc., Vancouver, BC), and RosetteSep (StemCell Technologies). A skilled artisan will recognize that other methodologies and kits may also be used to enrich or positively select desired cell populations. Dynabeads® Epithelial Enrich contains magnetic beads coated with mAbs specific for two glycoprotein membrane antigens expressed on normal and neoplastic epithelial tissues. The coated beads may be added to a sample and the sample then applied to a magnet, thereby capturing the cells bound to the beads. The unwanted cells are washed away and the magnetically isolated cells eluted from the beads and used in further analyses.

RosetteSep can be used to enrich cells directly from a blood sample and consists of a cocktail of tetrameric antibodies that targets a variety of unwanted cells

and crosslinks them to glycophorin A on red blood cells (RBC) present in the sample, forming rosettes. When centrifuged over Ficoll, targeted cells pellet along with the free RBC. The combination of antibodies in the depletion cocktail determines which cells will be removed and consequently which cells will be recovered. Antibodies that are available include, but are not limited to: CD2, CD3, CD4, CD5, CD8, CD10, CD11b, CD14, CD15, CD16, CD19, CD20, CD24, CD25, CD29, CD33, CD34, CD36, CD38, CD41, CD45, CD45RA, CD45RO, CD56, CD66B, CD66e, HLA-DR, IgE, and TCRαβ.

Additionally, it is contemplated in the present invention that mAbs specific for colon tumor antigens can be generated and used in a similar manner. For example, mAbs that bind to tumor-specific cell surface antigens may be conjugated to magnetic beads, or formulated in a tetrameric antibody complex, and used to enrich or positively select metastatic colon tumor cells from a sample. Once a sample is enriched or positively selected, cells may be lysed and RNA isolated. RNA may then be subjected to RT-PCR analysis using colon tumor-specific primers in a real-time PCR assay as described herein. One skilled in the art will recognize that enriched or selected populations of cells may be analyzed by other methods (*e.g. in situ* hybridization or flow cytometry).

In another embodiment, the compositions described herein may be used as markers for the progression of cancer. In this embodiment, assays as described above for the diagnosis of a cancer may be performed over time, and the change in the level of reactive polypeptide(s) or polynucleotide(s) evaluated. For example, the assays may be performed every 24-72 hours for a period of 6 months to 1 year, and thereafter performed as needed. In general, a cancer is progressing in those patients in whom the level of polypeptide or polynucleotide detected increases over time. In contrast, the cancer is not progressing when the level of reactive polypeptide or polynucleotide either remains constant or decreases with time.

Certain *in vivo* diagnostic assays may be performed directly on a tumor. One such assay involves contacting tumor cells with a binding agent. The bound binding agent may then be detected directly or indirectly via a reporter group. Such

binding agents may also be used in histological applications. Alternatively, polynucleotide probes may be used within such applications.

As noted above, to improve sensitivity, multiple tumor protein markers may be assayed within a given sample. It will be apparent that binding agents specific for different proteins provided herein may be combined within a single assay. Further, multiple primers or probes may be used concurrently. The selection of tumor protein markers may be based on routine experiments to determine combinations that results in optimal sensitivity. In addition, or alternatively, assays for tumor proteins provided herein may be combined with assays for other known tumor antigens.

The present invention further provides kits for use within any of the above diagnostic methods. Such kits typically comprise two or more components necessary for performing a diagnostic assay. Components may be compounds, reagents, containers and/or equipment. For example, one container within a kit may contain a monoclonal antibody or fragment thereof that specifically binds to a tumor protein. Such antibodies or fragments may be provided attached to a support material, as described above. One or more additional containers may enclose elements, such as reagents or buffers, to be used in the assay. Such kits may also, or alternatively, contain a detection reagent as described above that contains a reporter group suitable for direct or indirect detection of antibody binding.

Alternatively, a kit may be designed to detect the level of mRNA encoding a tumor protein in a biological sample. Such kits generally comprise at least one oligonucleotide probe or primer, as described above, that hybridizes to a polynucleotide encoding a tumor protein. Such an oligonucleotide may be used, for example, within a PCR or hybridization assay. Additional components that may be present within such kits include a second oligonucleotide and/or a diagnostic reagent or container to facilitate the detection of a polynucleotide encoding a tumor protein.

The following Examples are offered by way of illustration and not by way of limitation.

EXAMPLES

EXAMPLE 1

IDENTIFICATION OF COLON TUMOR PROTEIN cDNAS

This Example illustrates the identification of cDNA molecules encoding colon tumor proteins using PCR-based cDNA subtraction methodology.

A modification of the Clontech (Palo Alto, CA) PCR-Select™ cDNA subtraction methodology was employed to obtain cDNA populations enriched in cDNAs derived from transcripts that are differentially expressed in colon tumor samples. By this methodology, mRNA populations were isolated from colon tumor and metastatic tumor samples ("tester" mRNA) as well as from normal tissues, such as brain, pancreas, bone marrow, liver, heart, lung, stomach and small intestine ("driver" mRNA). From the tester and driver mRNA populations, cDNA was synthesized by standard methodology. *See, e.g.*, Ausubel, F.M. et al., *Short Protocols in Molecular Biology* (4^{th} ed., John Wiley and Sons, Inc., 1999).

The subtraction steps were performed using a PCR-based protocol that was modified to generate fragments larger than would be derived by the Clontech methodology. By this modified protocol, the tester and driver cDNAs were separately digested with five restriction endonucleases (Mlu I, Msc I, Pvu II, Sal I and Stu I) each of which recognize a unique 6-base pair nucleotide sequence. This digestion resulted in an average cDNA size of 600 bp, rather than the average size of 300 bp that results from digestion with Rsa I according to the Clontech methodology. This modification did not affect the ultimate subtraction efficiency.

Following the restriction digestion, adapter oligonucleotides having unique nucleotide sequences were ligated onto the 5' ends of the tester cDNAs; adapter oligonucleotides were not ligated onto the driver cDNAs. The tester and driver cDNAs were subsequently hybridized one to the other using an excess of driver cDNA. This hybridization step resulted in populations of (a) unhybridized tester cDNAs, (b) tester cDNAs hybridized to other tester cDNAs, (c) tester cDNAs hybridized to driver cDNAs, (d) unhybridized driver cDNAs and (e) driver cDNAs hybridized to driver cDNAs.

Tester cDNAs hybridized to other tester cDNAs were selectively amplified by a polymerase chain reaction (PCR) employing primers complementary to the ligated adapters. Because only tester cDNAs were ligated to adapter sequences, neither unhybridized tester or driver cDNAs, tester cDNAs hybridized to driver cDNAs nor driver cDNAs hybridized to driver cDNAs were amplified using adapter specific oligonucleotides. The PCR amplified tester cDNAs were cloned into the pCR2.1 plasmid vector (Invitrogen; Carlsbad, CA) to create a libraries enriched in differentially expressed colon tumor antigen and colon metastatic tumor antigen specific cDNAs.

Three thousand clones from the pCR2.1 tumor antigen cDNA libraries were randomly selected and used to obtain clones for microarray analysis (performed by Rosetta; Seattle, WA) and nucleotide sequencing. The cDNA insert from each pCR2.1 clone was PCR amplified as follows. Briefly, 0.5 µl of glycerol stock solution was added to 99.5 µl of PCR mix containing 80 µl H2O, 10 µl 10X PCR Buffer, 6 µl $MgCl_2$, 1 µl 10 mM dNTPs, 1 µl 100 mM M13 forward primer (CACGACGTTGTAAAACGACGG),, 1 µl 100 mM M13 reverse primer (CACAGGAAACAGCTATGACC), and 0.5 µl 5 u/ml Taq DNA polymerase. The M13 forward and reverse primers used herein were obtained from Operon Technologies (Alameda, CA). The PCR amplification was performed for thirty cycles under the following conditions: 95°C for 5 minutes, 92°C for 30 seconds, 57°C for 40 seconds, 75°C for 2 minutes and 75°C for 5 minutes.

mRNA expression levels for representative clones were determined using microarray technology in colon tumor tissues (n=25), normal colon tissues (n=6), kidney, lung, liver, brain, heart, esophagus, small intestine, stomach, pancreas, adrenal gland, salivary gland, resting PBMC, activated PBMC, bone marrow, dendritic cells, spinal cord, blood vessels, skeletal muscle, skin, breast and fetal tissues. An exemplary methodology for performing the microarray analysis is described in Schena *et al.*, *Science* 270:467-470. The number of tissue samples tested in each case was one (n=1), except where specifically noted above; additionally, all the above-mentioned tissues were derived from humans.

The PCR amplification products were dotted onto slides in an array format, with each product occupying a unique location in the array. mRNA was

extracted from the tissue sample to be tested, and fluorescent-labeled cDNA probes were generated by reverse transcription, according to standard methodology, in the presence of fluorescent nucleotides ψ5 and ψ3. *See, e.g.*, Ausubel, et al., *supra* for exemplary reaction conditions for performing the reverse transcription reaction; ψ5 and ψ3 fluorescent labeled nucleotides may be obtained, *e.g.*, from Amersham Pharmacia (Uppsala, Sweden) or NEN® Life Science Products, Inc. (Boston, MA). The microarrays were probed with the fluorescent-labeled cDNAs, the slides were scanned and fluorescence intensity was measured. Genetic MicroSystems instrumentation for preparing the cDNA microarrays and for measuring fluorescence intensity is available from Affymetrix (Santa Clara, CA).

An elevated fluorescence intensity in a microarray sector probed with cDNA probes obtained from a colon tumor or colon metastatic tumor tissue as compared to the fluorescence intensity in the same sector probed with cDNA probes obtained from a normal tissue indicates a tumor antigen gene that is differentially expressed in colon tumor or colon metastatic tumor tissue.

Clones disclosed herein as SEQ ID NOs: 1-234 and described in Tables 2-4 were identified from the PCR subtracted differential colon tumor and colon metastatic tumor cDNA libraries by the microarray based methodology. Of these 234 clones, those corresponding to SEQ ID NOs: 1, 6, 18-20, 27, 30, 37, 40, 57, 65, 81, 82, 86, 88, 91, 95, 96, 106, 107, 117, 121, 123, 126, 130, 148, 150, 152, 155, 157, 159, 161, 174, 175, 180, 182, 187, 190, 191, 192, 203, 204 and 209 showed no significant similarity to known sequences in Genbank.

TABLE 2

cDNA SEQUENCES SHOWING NO SIGNIFICANT SIMILARITY TO SEQUENCE IN GENBANK

Clone	SEQ ID NO.	EST	Element (384)	Element (96)	Ratio	Median Signal 1	Median Signal 2	96 Well Location
54172	1	Parathyroid/breast	p0022r16c12	R0085 H6	3.24	0.276	0.085	5G12
54034	6	Ovarian	p0018r08c10	R0067 H5	2.24	0.179	0.08	4D6
53949	18	Colon/pancreatic islet	p0016r15c12	R0061 F6	2.32	0.145	0.062	3E 5
53898	19	Colon/Gastric	p0016r01c14	R0058 B7	4.43	0.423	0.095	3A2
54069	20	Prostate/colon	p0019r03c02	R0070 F1	2.5	0.136	0.054	4G5
54089	27	Colon/HCC cell line	p0019r14c18	R0073 D9	2.97	0.096	0.032	5A1
54181	30	Br/Li/Ut/Pr	p0023r09c19	R0088 A10	2.85	0.264	0.092	5H9
54147	37	Colon only	p0021r12c01	R0080 G1	2.05	0.132	0.064	5E 11
54039	40	Ovary	p0018r09c06	R0068 B3	2.03	0.185	0.091	4D11
54059	57	Novel	p0018r13c20	R0069 B10	2.02	0.089	0.044	4F7
54141	65	HCC cell line/colon/testis	p0021r07c03	R0079 E2	2.35	0.106	0.045	5E 5
54120	81	Novel	p0020r11c07	R0076 E4	2.02	0.087	0.043	5C8
54145	82	Ut/Plac/Br/Pr	p0021r11c01	R0080 E1	2.5	0.147	0.059	5E 9
54152	86	Ut/Lu/Co/Pancreatic islet	p0021r14c23	R0081 C12	2.14	0.141	0.066	5F4
54146	88	Br/Co/melanocyte	p0021r11c19	R0080 E10	2.07	0.097	0.047	5E 10
54020	91	Fetal liver/heart	p0017r16c12	R0065 H6	2.18	0.133	0.061	4C4
54161	95	Fetal liver spleen	p0022r05c16	R0083 B8	2.07	0.083	0.04	5G1
54162	96	Lot EST	p0022r05c22	R0083 B11	3.74	0.205	0.055	5G2
54098	106	Lot EST	p0020r02c05	R0074 C3	2.06	0.064	0.031	5A10
54173	107	Co/Pan/Kid/Liver	p0022r16c23	R0085 G12	2.62	0.14	0.053	5H1

Clone	SEQ ID NO.	EST	Element (384)	Element (96)	Ratio	Median Signal 1	Median Signal 2	96 Well Location
54183	117	Co/Brn/Ut/Lu	p0023r10c20	R0088 D10	2.8	0.092	0.033	5H11
53918	121	Infant brain/breast	p0016r07c15	R0059 E8	2.06	0.104	0.051	3B10
53910	123	Co/Ut	p0016r05c11	R0059 A6	2.01	0.098	0.049	3B2
53917	126	Infant brain/gall bladder	p0016r07c02	R0059 F1	2	0.102	0.051	3B9
53999	130	Kid/Thymus/Co	p0017r12c08	R0064 H4	2.75	0.269	0.098	4A7
54074	148	Pr	p0019r04c04	R0070 H2	2	0.198	0.099	4G10
53961	150	Novel	p0017r03c06	R0062 F3	3.45	0.069	0.02	3F5
53933	152	Lot EST	p0016r10c21	R0060 C11	2.64	0.14	0.053	3D1
53924	155	Novel	p0016r08c11	R0059 G6	3.14	0.144	0.046	3C4
54068	157	Lot EST	p0019r01c12	R0070 B6	2.01	0.182	0.091	4G4
53959	159	Germinal center B cell	p0017r03c01	R0062 E1	2.01	0.042	0.021	3F3
53931	161	Pr/Lu	p0016r10c17	R0060 C9	2.41	0.152	0.063	3C11
54091	174	Kid/Stomach	p0019r15c06	R0073 F3	2.1	0.076	0.036	5A3
54013	175	Fetal tissues/testis	p0017r15c03	R0065 E2	2.32	0.183	0.079	4B9
53963	180	Lot EST	p0017r03c12	R0062 F6	2.59	0.256	0.099	3F7
54067	182	Lot EST	p0018r16c20	R0069 H10	4.8	0.347	0.072	4G3
53966	187	Infant brain	p0017r04c07	R0062 G4	2.08	0.119	0.057	3F10
54094	190	Co/Fetal retina	p0019r16c01	R0073 G1	2.11	0.149	0.071	5A6
53977	191	1887043	p0017r05c12	R0063 B6	2.35	0.164	0.07	3G9
54123	192	Infant brain/multiple scler	p0020r15c04	R0077 F2	2.01	0.091	0.045	5C11
54016	203	Novel	p0017r15c16	R0065 F8	2.04	0.113	0.055	4B12
54018	204	Br/Co	p0017r15c23	R0065 E12	3.48	0.203	0.058	4C2
53988	209	Kid/Co/Fetal brain	p0017r08c20	R0063 H10	2.88	0.117	0.041	3H8

TABLE 3

SEQUENCES WITH SOME DEGREE OF SIMILARITY TO SEQUENCES IN GENBANK WITH NO KNOWN FUNCTION

Clone	SEQ ID NO.	Genbank	EST	Element (384)	Element (96)	Ratio	Median Signal 1	Median Signal 2	96 Well Location
54104	2	PAC 75N13 on chromosome Xq21.1	Colon only	p0020r03c18	R0074 F9	2.15	0.098	0.045	5B4
54149	5	cDNA FLJ10461 fis, clone NT2RP1001482	Ovarian	p0021r13c12	R0081 B6	2.5	0.068	0.027	5F1
53948	8	12p12 BAC RPCI11-267J23	Testis/colon/liver	p0016r15c11	R0061 E6	2.05	0.147	0.072	3E 4
54026	9	Clone 164F3 on chromosome Xq21.33-23	Fetal liver/lung/colon	p0018r04c10	R0066 H5	2	0.125	0.062	4C10
54174	17	PAC clone RP1-170O19 from 7p15-p21	Colon only	p0023r03c09	R0086 E5	2.63	0.221	0.084	5H2

Clone	SEQ ID NO.	Genbank	EST	Element (384)	Element (96)	Ratio	Median Signal 1	Median Signal 2	96 Well Location
54048	21	cDNA FLJ20676 fis, clone KAIA4294	Pancreatic islet/prostate	p0018r11c17	R0068 E9	5.15	0.315	0.061	4E 8
54031	22	Chromosome 17, clone hRPC.1171_I_10	Co/Pr/Ov/Ut	p0018r07c23	R0067 E12	4.66	0.454	0.098	4D3
54079	31	PAC 75N13 on chromosome Xq21.1	Co/Gas	p0019r06c18	R0071 D9	3.04	0.199	0.066	4H3
54160	33	Clone 146H21 on chromosome Xq22	Colon only	p0022r05c08	R0083 B4	3.7	0.215	0.058	5F12
54078	35	PAC 75N13 on chromosome Xq21.1	Colon only	p0019r06c09	R0071 C5	2.79	0.145	0.052	4H2
54037	41	Constitutive fragile region FRA3B sequence 90%	Pancreatic islet/colon	p0018r08c24	R0067 H12	2.37	0.128	0.054	4D9

Clone	SEQ ID NO.	Genbank	EST	Element (384)	Element (96)	Ratio	Median Signal 1	Median Signal 2	96 Well Location
54052	51	cDNA FLJ10610 fis, clone NT2RP2005293	Novel	p0018r12c21	R0068 G11	2.36	0.072	0.031	4E 12
54124	63	Clone RP1-39G22 on chromosome 1p32.1-34.3	Kid/Ut/Infant brain	p0020r16c10	R0077 H5	2.07	0.149	0.072	5C12
54065	69	cDNA FLJ10969 fis, clone PLACE1000909	Kid/Ut	p0018r15c19	R0069 E10	2.36	0.193	0.082	4G1
54060	70	BAC clone 215O12	Pancreatic islet	p0018r14c16	R0069 D8	2.15	0.099	0.046	4F8
54136	78	KIAA1077 protein	Bt/Pr/Ut	p0021r04c24	R0078 H12	2.27	0.112	0.049	5D12
54140	80	PAC 454G6 on chromosome 1q24	Pan/HeLa cell/Ut	p0021r06c08	R0079 D4	2.17	0.062	0.029	5E 4
54117	83	KIAA0152	Ut/Co/Br/Lu	p0020r10c13	R0076 C7	2.02	0.063	0.031	5C5
54159	90	cDNA DKFZp586O0118	Lot	p0022r04c08	R0082 H4	2.64	0.159	0.06	5F11

Clone	SEQ ID NO.	Genbank	EST	Element (384)	Element (96)	Ratio	Median Signal 1	Median Signal 2	96 Well Location
54030	94	CGI-151/KIAA0992 protein	Endothelial cell/Sk Musc	p0018r06c22	R0067 D11	2.02	0.154	0.076	4D2
54133	101	cDNA DKFZp586I2022	Lu/Co/Ut	p0021r04c02	R0078 H1	2.63	0.136	0.052	5D9
54131	104	cDNA FLJ10549 fis, clone NT2RP2001976	Ut/GC/Pr	p0021r03c08	R0078 F4	2.03	0.083	0.041	5D7
54122	105	cDNA DKFZp434C0523	Embryo/fetal brain	p0020r12c04	R0076 H2	2.36	0.224	0.095	5C10
54179	110	cDNA FLJ10610 fis, clone NT2RP2005293	Thymus/fetal heart	p0023r08c18	R0087 H9	2.13	0.089	0.042	5H7
54027	116	cDNA FLJ10884 fis, clone NT2RP4001950	GC/testis	p0018r05c06	R0067 B3	2.15	0.181	0.084	4C11
54106	119	KIAA1289	Fetal tissue/melanocyte	p0020r04c19	R0074 G10	2.09	0.104	0.05	5B6

Clone	SEQ ID NO.	Genbank	EST	Element (384)	Element (96)	Ratio	Median Signal 1	Median Signal 2	96 Well Location
53904	122	Chromosome 17, clone hRPK.692_E_18	Co/fetal/placenta	p0016r03c15	R0058 E8	4.59	0.445	0.097	3A8
53903	124	cDNA FLJ10823 fis, clone NT2RP4001080	Colon only	p0016r03c12	R0058 F6	2.08	0.111	0.053	3A7
53928	133	citb_338_f_24, complete sequence	Ut/infant brain	p0016r09c19	R0060 A10	3.14	0.166	0.053	3C8
53930	139	Chromosome 19	6882084/6893421	p0016r10c04	R0060 D2	2.35	0.127	0.054	3C10
54005	143	Chromosome 5 clone CTC-436P18	GCB/infant brain	p0017r12c22	R0064 H11	2.07	0.132	0.064	4B1
54083	146	12q24 PAC RPCI1-261P5	Novel	p0019r08c18	R0071 H9	2.12	0.057	0.027	4H7
54105	149	Clone RP4-621F18 on chromosome 1p11.4-21.3	Total fetus/fetal liver	p0020r04c18	R0074 H9	2.46	0.095	0.039	5B5
53906	154	cDNA FLJ10679 fis, clone NT2RP2006565	Lot EST	p0016r03c24	R0058 F12	2.04	0.13	0.064	3A10

Clone	SEQ ID NO.	Genbank	EST	Element (384)	Element (96)	Ratio	Median Signal 1	Median Signal 2	96 Well Location
53942	160	KIAA1050	Fetus/fetal lung	p0016r14c05	R0061 C3	2.02	0.067	0.033	3D10
53935	162	cDNA FLJ11127 fis, clone PLACE1006225	Co/Pan/Ov/Ut	p0016r11c08	R0060 F4	2.77	0.19	0.069	3D3
54000	165	KIAA0965	Fetus/Co/Ut	p0017r12c09	R0064 G5	2.12	0.149	0.07	4A8
53953	169	cDNA DKFZp586H0519	Ovary/fetal brain	p0016r15c24	R0061 F12	2.49	0.141	0.057	3E 9
53945	173	cDNA FLJ20127 fis, clone COL06176	Novel	p0016r14c20	R0061 D10	2.21	0.108	0.049	3E 1
53987	178	Clone RP1-155G6 on chromosome 20	HeLa/placenta/testis	p0017r08c16	R0063 H8	2.05	0.159	0.078	3H7
54057	183	PAC RPCI-1 133G21 map 21q11.1 region D21S190	Novel	p0018r13c11	R0069 A6	2.11	0.091	0.043	4F5
53960	193	BAC clone RG083M05 from 7q21-7q22	Subtracted Hippocampus	p0017r03c02	R0062 F1	2.48	0.07	0.028	3F4

Clone	SEQ ID NO.	Genbank	EST	Element (384)	Element (96)	Ratio	Median Signal 1	Median Signal 2	96 Well Location
53976	194	Human STS WI-14644		p0017r05c09	R0063 A5	2.53	0.243	0.096	3G8
54081	199	PAC RPCI-1 133G21 map 21q11.1 region D21S190	Colon only	p0019r07c10	R0071 F5	4.66	0.225	0.048	4H5
54082	200	PAC clone RP5-1185I7 from 7q11.23-q21	GCB/total fetus	p0019r07c16	R0071 F8	2.38	0.105	0.044	4H6
53992	202	cDNA FLJ20673 fis, clone KAIA4464	Kid/GCB/Co	p0017r11c08	R0064 F4	2.03	0.128	0.063	3H12
53973	206	KIAA0715	Colon/Brain	p0017r04c24	R0062 H12	4.39	0.196	0.045	3G5
53982	208	KIAA1225	Lym/Co	p0017r06c24	R0063 D12	2.22	0.107	0.048	3H2
53991	211	cDNA FLJ20171 fis, clone COL09761	Lu/Ut/Ct	p0017r10c21	R0064 C11	2.81	0.062	0.022	3H11

TABLE 4

cDNA SEQUENCES WITH SOME DEGREE OF SIMILARITY TO KNOWN SEQUENCES IN GENBANK

Clone	SEQ ID NO.	Genbank	EST	Element (384)	Element (96)	Ratio	Median Signal 1	Median Signal 2	96 Well Location
53978	3	Glutamine:fructose-6-phosphate amidotransferase		p0017r05c14	R0063 B7	3.24	0.182	0.056	3G10
54184	4	Colon Kruppel-like factor		p0023r10c22	R0088 D11	3.55	0.222	0.062	5H12
54085	7	Human beta 2 gene		p0019r11c24	R0072 F12	2.08	0.184	0.089	4H9
53907	10	Lysyl hydroxylase isoform 2		p0016r04c04	R0058 H2	2.25	0.218	0.097	3A11
54066	11	Mucin 11		p0018r15c23	R0069 E12	3.87	0.222	0.057	4G2
54017	12	Mucin 11		p0017r15c20	R0065 F10	5.21	0.241	0.046	4C1
54006	13	Mucin 11		p0017r13c10	R0065 B5	3.97	0.246	0.062	4B2
53962	14	Epiregulin (EGF family)		p0017r03c09	R0062 E5	2.61	0.083	0.032	3F6
54028	15	Mucin 12		p0018r05c15	R0067 A8	2.14	0.068	0.032	4C12
54166	16	E1A enhancer binding protein		p0022r10c04	R0084 D2	2.5	0.226	0.09	5G6

Clone	SEQ ID NO.	Genbank	EST	Element (384)	Element (96)	Ratio	Median Signal 1	Median Signal 2	96 Well Location
54154	23	Alpha topoisomerase truncated form		p0021r15c12	R0081 F6	3.22	0.315	0.098	5F6
54009	24	Cytokeratin 20		p0017r14c11	R0065 C6	4.07	0.185	0.045	4B5
54070	25	Erythroblastosis virus oncogene homolog 2		p0019r03c03	R0070 E2	2.05	0.172	0.084	4G6
53998	26	Polyadenylate binding protein II		p0017r12c07	R0064 G4	3.73	0.368	0.099	4A6
54182	28	Transforming growth factor-beta induced gene product		p0023r10c07	R0088 C4	3.14	0.21	0.067	5H10
53989	29	GDP-mannose 4,6 dehydratase		p0017r08c24	R0063 H12	3.77	0.259	0.069	3H9
54114	32	Mus fork head transcription factor gene 92%	Kid/Co/Lu/Ut/Pr	p0020r09c13	R0076 A7	3.39	0.185	0.055	5C2

Clone	SEQ ID NO.	Genbank	EST	Element (384)	Element (96)	Ratio	Median Signal 1	Median Signal 2	96 Well Location
54168	34	Glutamine:fructose-6-phosphate amidotransferase		p0022r15c16	R0085 F8	2.4	0.224	0.093	5G8
53900	36	Intestinal peptide-associated transporter HPT-1		p0016r03c01	R0058 E1	2.11	0.114	0.054	3A4
54033	38	Human proteinase activated receptor-2		p0018r08c07	R0067 G4	2.89	0.143	0.049	4D5
54022	39	GalNAc-T3 gene		p0017r16c21	R0065 G11	2.54	0.193	0.076	4C6
54129	42	CD24 signal transducer gene		p0021r02c15	R0078 C8	2.5	0.239	0.096	5D5
54054	43	Human c-myb gene		p0018r13c02	R0069 B1	3.15	0.282	0.089	4F2
54055	44	Pyrroline-5-carboxylate synthase long form		p0018r13c03	R0069 A2	2.01	0.116	0.058	4F3
54046	45	Human zinc finger protein ZNF139		p0018r11c11	R0068 E6	2.39	0.179	0.075	4E 6

Clone	SEQ ID NO.	Genbank	EST	Element (384)	Element (96)	Ratio	Median Signal 1	Median Signal 2	96 Well Location
54047	46	Gene for membrane cofactor protein		p0018r11c16	R0068 F8	3.09	0.196	0.063	4E 7
54040	47	Colon Kruppel-like factor		p0018r09c08	R0068 B4	5.44	0.377	0.069	4D12
54035	48	Human capping protein alpha subunit isoform 1		p0018r08c16	R0067 H8	2.17	0.157	0.072	4D7
54130	49	Ig lambda-chain		p0021r02c19	R0078 C10	2.41	0.076	0.032	5D6
54045	50	Protein tyrosine kinase	Placenta/Liver/testis	p0018r10c22	R0068 D11	2.15	0.148	0.069	4E 5
54050	52	Human microtubule-associated protein 7		p0018r11c24	R0068 F12	2.51	0.171	0.068	4E 10
54051	53	Human retinoblastoma susceptibility protein		p0018r12c20	R0068 H10	2.02	0.172	0.085	4E 11
54178	54	Human reticulocalbin		p0023r06c09	R0087 C5	2.02	0.127	0.063	5H6

Clone	SEQ ID NO.	Genbank	EST	Element (384)	Element (96)	Ratio	Median Signal 1	Median Signal 2	96 Well Location
54148	55	Translation initiation factor eIF3 p36 subunit		p0021r13c01	R0081 A1	2.67	0.18	0.067	5E 12
54058	56	Human apurinic/apyrimidinic endonuclease		p0018r13c12	R0069 B6	2.31	0.105	0.045	4F6
54126	58	Human integral transmembrane protein 1		p0021r01c05	R0078 A3	2.31	0.117	0.051	5D2
54127	59	Human serine kinase		p0021r01c15	R0078 A8	2.31	0.171	0.074	5D3
54049	60	Human CGI-44 protein		p0018r11c18	R0068 F9	2.24	0.191	0.085	4E 9
54056	61	HADH/NADPH thyroid oxidase p138-tox protein		p0018r13c05	R0069 A3	2.41	0.149	0.062	4F4
54064	62	Human peptide transporter (TAP1) protein		p0018r15c13	R0069 E7	2.96	0.104	0.035	4F12

Clone	SEQ ID NO.	Genbank	EST	Element (384)	Element (96)	Ratio	Median Signal 1	Median Signal 2	96 Well Location
54063	64	Transforming growth factor-beta induced gene product		p0018r15c10	R0069 F5	3.89	0.298	0.077	4F11
54119	66	Cytokeratin 8		p0020r11c02	R0076 F1	5.56	0.193	0.035	5C7
54111	67	Human coat protein gamma-cop		p0020r07c24	R0075 F12	2.05	0.076	0.037	5B11
54121	68	Bumetanide-sensitive Na-K-Cl cotransporter		p0020r11c20	R0076 F10	3.76	0.358	0.095	5C9
54125	71	Autoantigen calreticulin		p0020r16c20	R0077 H10	2.09	0.16	0.076	5D1
54143	72	Human hepatic squalene synthetase		p0021r09c21	R0080 A11	2.16	0.132	0.061	5E 7
54139	73	Human RAD21 homolog		p0021r05c12	R0079 B6	2.26	0.06	0.026	5E 3
54137	74	Human MHC class II HLA-DR-alpha		p0021r05c08	R0079 B4	2.16	0.097	0.045	5E 1
54044	75	Human Claudin-7		p0018r10c12	R0068 D6	5.03	0.277	0.055	4E 4

Clone	SEQ ID NO.	Genbank	EST	Element (384)	Element (96)	Ratio	Median Signal 1	Median Signal 2	96 Well Location
54042	76	Ribosome protein S6 kinase 1		p0018r09c20	R0068 B10	3.56	0.116	0.033	4E 2
54043	77	CO-029 tumor associated antigen	Colon/Pancreatic	p0018r10c11	R0068 C6	2.65	0.18	0.068	4E 3
54157	79	Human lipocortin II		p0022r02c18	R0082 D9	3.84	0.265	0.069	5F9
54116	84	Tumor antigen L6		p0020r10c03	R0076 C2	2	0.105	0.052	5C4
54151	85	UDP-N-acetylglucosamine transporter		p0021r14c15	R0081 C8	2.35	0.093	0.04	5F3
54115	87	Cystine/glutamate transporter		p0020r09c16	R0076 B8	2.05	0.033	0.016	5C3
54155	89	GAPDH		p0022r01c04	R0082 B2	4.23	0.417	0.099	5F7
54169	92	Neutrophil lipocalin		p0022r15c24	R0085 F12	2.74	0.216	0.079	5G9
54167	93	Nuclear matrix protein NRP/B		p0022r13c20	R0085 B10	2.38	0.084	0.035	5G7
54163	97	Poly A binding protein		p0022r06c14	R0083 D7	3.28	0.262	0.08	5G3

Clone	SEQ ID NO.	Genbank	EST	Element (384)	Element (96)	Ratio	Median Signal 1	Median Signal 2	96 Well Location
54164	98	Ribosome protein L13		p0022r08c13	R0083 G7	2.01	0.105	0.052	5G4
54132	99	Human alpha enolase		p0021r03c13	R0078 E7	2.96	0.292	0.099	5D8
54112	100	Human E-1 enzyme		p0020r08c03	R0075 G2	2.06	0.097	0.047	5B12
54165	102	Human ZW10 interactor Zwint		p0022r09c22	R0084 B11	2.46	0.055	0.022	5G5
54158	103	Bumetanide-sensitive Na-K-Cl cotransporter		p0022r03c20	R0082 F10	2.61	0.241	0.092	5F10
54108	108	NADH-ubiquinone oxidoreductase NDUFS2 subunit		p0020r06c11	R0075 C6	2.07	0.105	0.051	5B8
54175	109	Phospholipase A2		p0023r04c03	R0086 G2	3.28	0.187	0.057	5H3
54177	111	Ig heavy chain variable region		p0023r05c08	R0087 B4	2.31	0.117	0.051	5H5
54170	112	Protein phosphatase 2C gamma		p0022r16c04	R0085 H2	2.03	0.136	0.067	5G10
54176	113	Cyclin protein		p0023r04c06	R0086 H3	2.12	0.165	0.078	5H4

Clone	SEQ ID NO.	Genbank	EST	Element (384)	Element (96)	Ratio	Median Signal 1	Median Signal 2	96 Well Location
54180	114	Transgelin 2 (predicted)		p0023r09c09	R0088 A5	2.21	0.166	0.075	5H8
53897	115	Human GalNAc-T3 gene		p0016r01c11	R0058 A6	2.46	0.179	0.073	3A1
54107	118	Alpha topoisomeras e truncated form		p0020r05c22	R0075 B11	2.64	0.108	0.041	5B7
53902	120	AD022 protein		p0016r03c04	R0058 F2	2.3	0.123	0.053	3A6
54004	127	Cytochrome P450 IIIA4 82%		p0017r12c21	R0064 G11	2.07	0.134	0.065	4A12
53913	128	CEA		p0016r05c23	R0059 A12	5.48	0.338	0.062	3B5
54134	129	Protein phosphatase (KAP1)		p0021r04c05	R0078 G3	2.05	0.138	0.067	5D10
53938	131	Alpha enolase		p0016r12c15	R0060 G8	3.04	0.299	0.098	3D6
53939	132	Histone deacetylase HD1		p0016r12c23	R0060 G12	2.37	0.17	0.072	3D7
53914	134	Human squalene epoxidase		p0016r06c03	R0059 C2	2.12	0.07	0.033	3B6

Clone	SEQ ID NO.	Genbank	EST	Element (384)	Element (96)	Ratio	Median Signal 1	Median Signal 2	96 Well Location
53915	135	Human aspartyl-tRNA-synthetase alpha-2 subunit		p0016r06c09	R0059 C5	2.02	0.121	0.06	3B7
54101	136	Gamma-actin		p0020r02c20	R0074 D10	2.91	0.21	0.072	5B1
53922	137	Human AP-mu chain family member mu1B		p0016r07c21	R0059 E11	2.07	0.161	0.078	3C2
54023	138	Human Cctg mRNA for chaperonin		p0018r02c21	R0066 C11	2.87	0.192	0.067	4C7
53921	140	Human MEGF7		p0016r07c20	R0059 F10	2.5	0.109	0.044	3C1
54002	141	Connexin 26		p0017r12c15	R0064 G8	2.13	0.133	0.063	4A10
54003	142	Human dipeptidyl peptidase IV		p0017r12c16	R0064 H8	2	0.081	0.04	4A11
53925	144	Human 2-oxoglutarate dehydrogenase		p0016r08c16	R0059 H8	2.7	0.167	0.062	3C5

Clone	SEQ ID NO.	Genbank	EST	Element (384)	Element (96)	Ratio	Median Signal 1	Median Signal 2	96 Well Location
53927	145	Rho guanine nucleotide-exchange factor		p0016r09c12	R0060 B6	2.13	0.194	0.091	3C7
53937	147	Human colon mucosa-associated mRNA	Normal colon	p0016r11c23	R0060 E12	2.89	0.153	0.053	3D5
53919	151	Human embryonic lung protein		p0016r07c16	R0059 F8	2.19	0.153	0.07	3B11
53972	153	Human leukocyte surface protein CD31		p0017r04c18	R0062 H9	2.08	0.052	0.025	3G4
54144	156	Poly A binding protein		p0021r09c24	R0080 B12	2.99	0.163	0.055	5E 8
53929	158	Cystic fibrosis transmembrane conductance regulator		p0016r10c02	R0060 D1	4.15	0.181	0.044	3C9
54099	163	Human set gene		p0020r02c07	R0074 C4	2.19	0.133	0.061	5A11
53943	164	Human pleckstrin 2		p0016r14c15	R0061 C8	3	0.155	0.052	3D11
54100	166	Tis11d gene		p0020r02c09	R0074 C5	2.2	0.183	0.083	5A12

Clone	SEQ ID NO.	Genbank	EST	Element (384)	Element (96)	Ratio	Median Signal 1	Median Signal 2	96 Well Location
53940	167	Cytokine (GRO-gamma)		p0016r13c17	R0061 A9	2.37	0.183	0.077	3D8
53941	168	Human p85Mcm mRNA		p0016r13c23	R0061 A12	2.25	0.09	0.04	3D9
54007	170	SOX9		p0017r13c19	R0065 A10	2.32	0.228	0.098	4B3
53950	171	VAV-like protein		p0016r15c14	R0061 F7	2.41	0.064	0.026	3E 6
53968	172	NF-E2 related factor 3		p0017r04c10	R0062 H5	2.19	0.1	0.046	3F12
54092	176	Human argininosucci nate synthetase		p0019r15c10	R0073 F5	2.73	0.199	0.073	5A4
54095	177	Human serine kinase		p0019r16c14	R0073 H7	2.57	0.126	0.049	5A7
53967	179	Human phospholipase C beta 4		p0017r04c08	R0062 H4	2.87	0.182	0.063	3F11
54032	181	VAV-3 protein		p0018r08c01	R0067 G1	2.16	0.096	0.044	4D4
54135	184	Calcium-binding protein S100P		p0021r04c13	R0078 G7	5.65	0.474	0.084	5D11
53969	185	Human leupaxin		p0017r04c14	R0062 H7	2.12	0.042	0.02	3G1

Clone	SEQ ID NO.	Genbank	EST	Element (384)	Element (96)	Ratio	Median Signal 1	Median Signal 2	96 Well Location
53970	186	VAV-3 protein		p0017r04c15	R0062 G8	2.9	0.123	0.042	3G2
53995	188	hnRNP type A/B protein		p0017r11c23	R0064 E12	2.31	0.106	0.046	4A3
54075	189	Human cell cycle control gene CDC2		p0019r04c06	R0070 H3	3.57	0.222	0.062	4G11
54096	195	Human glutaminyl-tRNA synthetase		p0019r16c15	R0073 G8	2.17	0.206	0.095	5A8
54110	196	Human 26S proteasome-associated pad 1 homolog		p0020r07c22	R0075 F11	2.37	0.187	0.079	5B10
53920	197	Human squalene epoxidase		p0016r07c18	R0059 F9	3	0.205	0.068	3B12
53979	198	Human nuclear chloride ion channel protein		p0017r05c16	R0063 B8	2.2	0.116	0.053	3G11
53986	201	Human ephrin		p0017r08c09	R0063 G5	2.15	0.212	0.099	3H6
53985	205	CD9 antigen		p0017r08c06	R0063 H3	3.2	0.315	0.099	3H5
54012	207	Cyclin B		p0017r14c19	R0065 C10	2.73	0.156	0.057	4B8

Clone	SEQ ID NO.	Genbank	EST	Element (384)	Element (96)	Ratio	Median Signal 1	Median Signal 2	96 Well Location
53990	210	Colon mucosa-associated mRNA		p0017r09c22	R0064 B11	2.27	0.116	0.051	3H10

EXAMPLE 2

C907P IS OVEREXPRESSED IN COLON TUMORS

Using the C907P cDNA sequence, which was discovered from the subtracted cDNA library and cDNA microarray discussed above, the Genbank database was searched. C907P matches with a known gene named Epiregulin (Genbank accession number D30783). Two gene-specific primers were synthesized, and used for PCR amplification to clone this gene from colon cDNAs. The amplified PCR product was sequenced to confirm its identity. Thus, C907P-Epiregulin is a gene up-regulated in colon cancer. PCR was performed under conditions of denaturing cDNA at 94°C for 1 minute, then 35 cycles of 94°C for 30 seconds, 60°C for 30 seconds, 72°C for 2 minutes. Proof-reading polymerase was used for the amplification. The cDNA templates used for the PCR were synthesized from colon tumor mRNA. The amplified products were cloned into the TA cloning vector and the sequences were determined. The C907P DNA sequence is shown in SEQ ID NO:234, and the amino acid sequence is shown in SEQ ID NO:235.

EXAMPLE 3

FULL LENGTH PCR AMPLIFICATION AND cDNA CLONING OF THE C915P COLON TUMOR ANTIGEN

The C915P cDNA sequence (SEQ ID NO:33; also referred to by clone identifier number 54160), discovered from the subtracted cDNA library and cDNA microarray discussed in Example 1, was used to search the Genbank database. C915P was found to have some degree of similarity to a known gene named superoxidegenerating oxidase Mox1 (Genbank accession number AF127763). Two gene-specific primers were designed according to the sequence deposited in Genbank in order to amplify the full-length cDNA. PCR was performed under conditions of denaturing cDNA at 94°C for 1 minute, then 35 cycles of 94°C for 30 second, 60°C for 30 second, 72°C for 2 minutes. Proofreading polymerase was used for the amplification. The cDNA templates used for the PCR were synthesized from colon tumor mRNA. The amplified products were cloned into the TA cloning vector (Invitrogen, Carlsbad, CA) and random clones sequenced by automatic DNA

sequencing to confirm identity. The full-length cDNA and amino acid sequence of C915P is set forth in SEQ ID NO:244 and 245, respectively.

Expression levels of C915P cDNA were further analyzed by real-time PCR. Using this analysis, C915P was confirmed to be overexpressed in colon tumors as compared to a panel of normal tissues. Moderate levels of expression were observed in normal colon tissues. Real-time PCR (*see* Gibson et al., *Genome Research 6*:995-1001, 1996; Heid et al., *Genome Research 6*:986-994, 1996) is a technique that evaluates the level of PCR product accumulation during amplification. This technique permits quantitative evaluation of mRNA levels in multiple samples. Briefly, mRNA was extracted from colon tumor and normal tissue and cDNA was prepared using standard techniques. Real-time PCR was performed using a Perkin Elmer/Applied Biosystems (Foster City, CA) 7700 Prism instrument. Matching primers and a fluorescent probe were designed for C915P using the primer express program provided by Perkin Elmer/Applied Biosystems (Foster City, CA). Optimal concentrations of primers and probe were initially determined and control (*e.g.*, β-actin) primers and probe were obtained commercially. To quantitate the amount of specific RNA in a sample, a standard curve was generated using a plasmid containing the C915P cDNA. Standard curves were generated using the Ct values determined in the real-time PCR, which are related to the initial cDNA concentration used in the assay. Standard dilutions ranging from $10\text{-}10^6$ copies of the C915P were generally sufficient. In addition, a standard curve was generated for the control sequence. This permitted standardization of initial RNA content of the tissue samples to the amount of control for comparison purposes.

EXAMPLE 4

PRODUCTION OF RA12-C915P-F3 RECOMBINANT PROTEIN IN *E. COLI*

C915P (also referred to as clone identifier 54160, and set forth in SEQ ID NOs:33 and 244 (cDNA), and 245 (amino acid)) has 6 transmembrane domains (TMs) with 3 extracellular loops (ED1, ED2, and ED3). The deletion recombinant protein, Ra12-C915P-f3 (set forth in SEQ ID NOs:236 (cDNA) and 237 (amino acid)),

is an N-terminal Ra12 fusion of recombinant, modified C915P in pCRX1 vector (EcoR I, Xho I).

Cloning Strategy for Ra12-C915P-f3:

Three sets of primers were designed that were used sequentially to delete two internal transmembrane domains and amplify a recombined internal region of C915P that was cut with EcoRI and XhoI and ligated in frame with Ra12 in the pCRX1 vector.

PCR#1 used primers AW157 and AW156 (SEQ ID NO:241 and 240, respectively) to amplify the entire construct, deleting TM4 - ID3 - TM5. The PCR product (C915P(minusTM4-ID3-TM5) PCR Blunt II TOPO backbone) was purified from agarose gel, ligated by T4 DNA Ligase and transformed into NovaBlue *E. coli* cells with the following standard protocol: the competent *E. coli* cells were thawed on ice, DNA (or ligation mixture) was added, the reaction mixed and incubated on ice for 5 minutes. The *E. coli* cells were heat-shocked at 42°C for 30 seconds, and left on ice for 2 minutes. Enriched growth media was added to the *E. coli* and they were grown at 37°C for 1 hour. The culture was plated on LB (plus appropriate antibiotics) and grown overnight at 37°C. The next day, several colonies were randomly selected for miniprep (Promega, Madison, WI) and were confirmed by DNA sequencing for correctly deleted region. This step was then repeated on a second region of C915P as described below.

PCR#2 used primers AW155 and AW154 (SEQ ID NOs:239 and 238, respectively) to delete TM2, using a confirmed clone from PCR#1 as template. The PCR product (C915P(minusTM2 / TM4-ID3-TM5) PCR Blunt II TOPO backbone) was purified, ligated and transformed using standard protocols into NovaBlue cells, yielding clones that were confirmed by sequencing for the correct deletion.

PCR#3 used primers AW158 and AW159 (SEQ ID NOs:242 and 243, respectively) to amplify the deleted, recombined three-part fusion protein of C915P, ED1 - ID2-TM3-ED2 - ED3, using the confirmed PCR#2 clone as template. PCR product from PCR#3 was purified and digested using EcoR I and Xho I for ligation into the pCRX1 vector (EcoR I, Xho I). The ligation mixture was transformed into NovaBlue cells by standard protocols, and several clones were selected for miniprep

and sequencing. UI#70526 was confirmed by DNA sequencing to be the correct pCRX1 Ra12-C915P-f3 construct.

Cloning Primers:

C915P-AW154 (SEQ ID NO:238): antisense cloning primer to delete TM2, 5'P—Primer Id9682: 5' P- TTTTCTTGTGTAGTAGTATTTGTCG.

C915P-AW155 (SEQ ID NO:239): sense cloning primer to delete TM2, 5'P—Id 9683: 5' P-TGTCGCAATCTGCTGTCCTTCC.

C915P-AW156 (SEQ ID NO:240): antisense cloning primer to delete TM4-TM5 region, 5'-P, --Primer Id 9684: 5' P- GCTGGTGAATGTCACATACTCC.

C915P-AW157 (SEQ ID NO:241): sense cloning primer to delete TM4-TM5 region, 5'-P – Id 9685: 5' P- CGGGGTCAAACAGAGGAGAG.

Ra12-C915P-F3-AW158 (SEQ ID NO:242): sense cloning primer for the fusion protein with EcoR I site Primer Id 9686: 5' gtcgaattcGATGCCTTCCTGAAATATGAGAAG.

Ra12-C915P-F3-AW159 (SEQ ID NO:243): antisense cloning primer for the fusion protein with stop and Xho I site – Primer Id 9687: 5' cacctcgagttaAGACTCAGGGGGATGCCCTTC.

<u>Protein Information for Ra12-C915P-f3:</u>

Molecular Weight 32429.45 Daltons

297 Amino Acids

28 Strongly Basic(+) Amino Acids (K,R)

27 Strongly Acidic(-) Amino Acids (D,E)

93 Hydrophobic Amino Acids (A,I,L,F,W,V)

86 Polar Amino Acids (N,C,Q,S,T,Y)

7.776 Isolectric Point

3.711 Charge at PH 7.0

<u>Protein Expression:</u>

Mini expression screens were performed to determine the optimal induction conditions for Ra12-C915P-f3. The best *E. coli* strain/culture conditions

were screened by transforming the expression construct into different hosts, then varying temperature, culture media and/or IPTG concentration after the inducer IPTG was added to the mid-log phase culture. The recombinant protein expression was then analyzed by SDS-PAGE and/or Western blot. *E. coli* expression hosts BLR (DE3) and HMS (DE3) (Novagen, Madison, WI) were tested in various culture conditions, with little full-length Ra12-C915P-f3 expression detected and Western blots showing some bands at unexpected molecular weights. Tuner (DE3) cells (Novagen, Madison, WI) were then tested with helper plasmids at various IPTG concentrations. Coomassie stained SDS-PAGE showed no induced band but Western blot confirmed a strong Ra12-C915P-f3 signal at 32kD probing with an anti-6xhis tag antibody. The most optimal expression for pCRX1 Ra12-C915P-f3 was found to be in the host strain Tuner (DE3) with a helper plasmid grown in Soy Terrific Broth media at 37°C induced with 1.0 mM IPTG at 37°C for 3hr.

EXAMPLE 5

PURIFICATION OF RA12-C915P-F3 RECOMBINANT FUSION PROTEIN FROM *E. COLI*

The clone C915P was found to be over-expressed in a majority of colon cancer tissues. For expression in *E. coli*, the construct Ra12-C915P-f3 (SEQ ID NO:236) was made as described in Example 4. This construct encodes a fusion protein consisting of an N-terminal 6x histidine tag followed by Ra12 and modified C915P (excluding 5 of 6 transmembrane domains) (SEQ ID NO:237). The 32.4kD protein was expressed in multiple large baffled shaker flasks containing 1L of Soy Terrific Broth media. The cultures were spun and cell pellets washed, respun and frozen for purification. After cell lysis, the recombinant protein was found in the insoluble inclusion body fraction. The inclusion body was thoroughly washed with buffered detergents multiple times, then the protein pellet was denatured, reduced and solubilized in buffered 8M Urea and Ra12-C915P-f3 protein was bound to a Ni-NTA affinity chromatography matrix. The matrix was washed to rinse away contaminating *E. coli* proteins and Ra12-C915P-f3 was subsequently eluted using high Imidazole concentration. The fractions containing Ra12-C915P-f3 were pooled and slowly dialyzed to allow for renaturation of the protein. The purified Ra12-C915P-f3 was then

filtered and quantified. SDS-PAGE analysis showed the elution pattern off the nickel column with the major band running at the expected weight of about 32kD. This was further confirmed by western blot using an anti-6x His tag antibody. The western blot also revealed evidence of dimers and tetramers of the recombinant. N-terminal sequencing confirmed purity of about 90%. Purified yield was about 2.5 mg/L induction.

Following is a detailed protocol of the production of purified Ra12-C915P-f3.

For the frozen bacterial cell pellet:

1. Thaw bacterial cell pellet from 1L induction on ice
2. Add 25ml sonication buffer (20mM Tris, 500mM NaCl) per liter of induction culture
3. Add 1 Complete protease inhibitor tablet and 2mM PMSF (Phenylmethylsulfonyl fluoride) to sonication buffer/pellet mix
4. Completely resuspend pellet with pipet
5. Add 0.5mg/ml lysozyme (made fresh from lyophilized lysozyme stored at –20°C)
6. Decant into a glass beaker + stir bar, gently stir at 4°C, 30 min
7. French Press 2 x 1100psi, keep on ice
8. Once lysis solution** has low viscosity, spin at 11000RPM, 30min, 4°C
9. Save supernatant** and pellet

For the pellet from step 9 above:

1. Wash pellet with 25ml 0.5% CHAPS (3-([3-Cholamidopropyl]dimethylammonio)-1-propanesulfonate) wash (20mM Tris (8.0), 500mM NaCl) ** by sonicating 2x15sec @15Watt
2. Spin at 11000RPM for 25min. Repeat 5x**
3. Repeat above steps 3 times with 0.5% DOC (Deoxycholic Acid) wash (20mM Tris (8.0), 500mM NaCl)

4. Resuspend pellet in pellet binding buffer (20mM Tris (8.0), 500mM NaCl, 8M Urea, 20mM Imidazole, 10mM β-Mercaptoethanol) with sonication
5. Equilibrate Ni ++ NTA (Nitrilotriacetic acid) resin (Qiagen, Valencia, CA) with pellet binding buffer, spin down and decant wash (use 4ml resin)
6. Add resin to resuspended pellet, stir at room temperature for 45min
7. Prepare column and buffers, rinse column with pellet binding buffer
8. Pour pellet/Ni resin into column, collect flow through (FT)**
9. Wash column with 30ml pellet binding buffer **
10. Wash column with 30ml pellet binding buffer with 0.5% DOC (Deoxycholic Acid)**
11. Wash column with 30ml pellet binding buffer
12. Elute with 5 x 5ml fractions of pellet binding buffer #1 (binding buffer +300mM Imidazole)**
13. Elute with 2 x 5ml fractions of pellet elution buffer #2 (binding buffer +300mM Imidazole, pH 4.5)**
14. Run SDS-PAGE to screen purification steps (western and coomassie stain)

**Save an aliquot at 4°C for each purification step to check on SDS-PAGE.

EXAMPLE 6

REAL-TIME PCR ANALYSIS OF COLON TUMOR CANDIDATE GENES

The first-strand cDNA to be used in the quantitative real-time PCR was synthesized from 20μg of total RNA that had been treated with DNase I (Amplification Grade, Gibco BRL Life Technology, Gaitherburg, MD), using Superscript Reverse Transcriptase (RT) (Gibco BRL Life Technology, Gaitherburg, MD). Real-time PCR

was performed with a GeneAmp™ 5700 sequence detection system (PE Biosystems, Foster City, CA). The 5700 system uses SYBR™ green, a fluorescent dye that only intercalates into double stranded DNA, and a set of gene-specific forward and reverse primers. The increase in fluorescence is monitored during the whole amplification process. The optimal concentration of primers was determined using a checkerboard approach and a pool of cDNAs from breast tumors was used in this process. The PCR reaction was performed in 25µl volumes that include 2.5µl of SYBR green buffer, 2µl of cDNA template and 2.5µl each of the forward and reverse primers for the gene of interest. The cDNAs used for RT reactions were diluted 1:10 for each gene of interest and 1:100 for the β-actin control. In order to quantitate the amount of specific cDNA (and hence initial mRNA) in the sample, a standard curve is generated for each run using the plasmid DNA containing the gene of interest. Standard curves were generated using the Ct values determined in the real-time PCR which were related to the initial cDNA concentration used in the assay. Standard dilution ranging from $20\text{-}2\times10^6$ copies of the gene of interest was used for this purpose. In addition, a standard curve was generated for β-actin ranging from 200fg-2000fg. This enabled standardization of the initial RNA content of a tissue sample to the amount of β-actin for comparison purposes. The mean copy number for each group of tissues tested was normalized to a constant amount of β-actin, allowing the evaluation of the over-expression levels seen with each of the genes.

Colon tumor candidate genes, C906P (SEQ ID NO:5), C907P (SEQ ID NO:234 (cDNA) and 235 (amino acid)), C911P (SEQ ID NO:21), C915P (SEQ ID NO:244 (cDNA) and 245 (amino acid)), C943P (SEQ ID NO:140), and C961P (SEQ ID NO:200), were analyzed by real-time PCR, as described above, using the short and extended colon panel. These genes were found to have increased mRNA expression in 30-50% of colon tumors. For C906P, slightly elevated expression was also observed in normal trachea, heart, and normal colon. For C907P, elevated expression was also observed in activated PBMC and slightly elevated expression in heart and normal colon. For C911P, slightly elevated expression was observed in pancreas. For C915P, no expression was observed in normal tissues except normal colon. For C943P, no expression was observed in normal tissues except normal colon. For C961P, some

expression was observed in trachea and normal colon. Collectively, the data indicate that these colon tumor candidate genes could be potential targets for immunotherapy and cancer diagnosis.

EXAMPLE 7

PEPTIDE PRIMING OF T-HELPER LINES

Generation of $CD4^+$ T helper lines and identification of peptide epitopes derived from tumor-specific antigens that are capable of being recognized by $CD4^+$ T cells in the context of HLA class II molecules, is carried out as follows:

Fifteen-mer peptides overlapping by 10 amino acids, derived from a tumor-specific antigen, are generated using standard procedures. Dendritic cells (DC) are derived from PBMC of a normal donor using GM-CSF and IL-4 by standard protocols. $CD4^+$ T cells are generated from the same donor as the DC using MACS beads (Miltenyi Biotec, Auburn, CA) and negative selection. DC are pulsed overnight with pools of the 15-mer peptides, with each peptide at a final concentration of 0.25 μg/ml. Pulsed DC are washed and plated at 1×10^4 cells/well of 96-well V-bottom plates and purified $CD4^+$ T cells are added at 1×10^5/well. Cultures are supplemented with 60 ng/ml IL-6 and 10 ng/ml IL-12 and incubated at 37°C. Cultures are restimulated as above on a weekly basis using DC generated and pulsed as above as antigen presenting cells, supplemented with 5 ng/ml IL-7 and 10 U/ml IL-2. Following 4 *in vitro* stimulation cycles, resulting $CD4^+$ T cell lines (each line corresponding to one well) are tested for specific proliferation and cytokine production in response to the stimulating pools of peptide with an irrelevant pool of peptides used as a control.

EXAMPLE 8

GENERATION OF TUMOR-SPECIFIC CTL LINES USING IN VITRO WHOLE-GENE PRIMING

Using *in vitro* whole-gene priming with tumor antigen-vaccinia infected DC (see, for example, Yee et al, *The Journal of Immunology*, 157(9):4079-86, 1996), human CTL lines are derived that specifically recognize autologous fibroblasts transduced with a specific tumor antigen, as determined by interferon-γ ELISPOT

analysis. Specifically, dendritic cells (DC) are differentiated from monocyte cultures derived from PBMC of normal human donors by growing for five days in RPMI medium containing 10% human serum, 50 ng/ml human GM-CSF and 30 ng/ml human IL-4. Following culture, DC are infected overnight with tumor antigen-recombinant vaccinia virus at a multiplicity of infection (M.O.I) of five, and matured overnight by the addition of 3 μg/ml CD40 ligand. Virus is then inactivated by UV irradiation. CD8+ T cells are isolated using a magnetic bead system, and priming cultures are initiated using standard culture techniques. Cultures are restimulated every 7-10 days using autologous primary fibroblasts retrovirally transduced with previously identified tumor antigens. Following four stimulation cycles, CD8+ T cell lines are identified that specifically produce interferon-γ when stimulated with tumor antigen-transduced autologous fibroblasts. Using a panel of HLA-mismatched B-LCL lines transduced with a vector expressing a tumor antigen, and measuring interferon-γ production by the CTL lines in an ELISPOT assay, the HLA restriction of the CTL lines is determined.

EXAMPLE 9

GENERATION AND CHARACTERIZATION OF ANTI-TUMOR ANTIGEN MONOCLONAL ANTIBODIES

Mouse monoclonal antibodies are raised against *E. coli* derived tumor antigen proteins as follows: Mice are immunized with Complete Freund's Adjuvant (CFA) containing 50 μg recombinant tumor protein, followed by a subsequent intraperitoneal boost with Incomplete Freund's Adjuvant (IFA) containing 10μg recombinant protein. Three days prior to removal of the spleens, the mice are immunized intravenously with approximately 50μg of soluble recombinant protein. The spleen of a mouse with a positive titer to the tumor antigen is removed, and a single-cell suspension made and used for fusion to SP2/O myeloma cells to generate B cell hybridomas. The supernatants from the hybrid clones are tested by ELISA for specificity to recombinant tumor protein, and epitope mapped using peptides that spanned the entire tumor protein sequence. The mAbs are also tested by flow

cytometry for their ability to detect tumor protein on the surface of cells stably transfected with the cDNA encoding the tumor protein.

From the foregoing it will be appreciated that, although specific embodiments of the invention have been described herein for purposes of illustration, various modifications may be made without deviating from the spirit and scope of the invention. Accordingly, the invention is not limited except as by the appended claims.

CLAIMS

What is Claimed:

1. An isolated polynucleotide comprising a sequence selected from the group consisting of:

(a) sequences provided in SEQ ID NO:1-234, 236, and 244;

(b) complements of the sequences provided in SEQ ID NO:1-234, 236, and 244;

(c) sequences consisting of at least 20 contiguous residues of a sequence provided in SEQ ID NO:1-234, 236, and 244;

(d) sequences that hybridize to a sequence provided in SEQ ID NO:1-234, 236, and 244, under moderately stringent conditions;

(e) sequences having at least 75% identity to a sequence of SEQ ID NO:1-234, 236, and 244;

(f) sequences having at least 90% identity to a sequence of SEQ ID NO:1-234, 236, and 244; and

(g) degenerate variants of a sequence provided in SEQ ID NO:1-234, 236, and 244.

2. An isolated polypeptide comprising an amino acid sequence selected from the group consisting of:

(a) sequences encoded by a polynucleotide of claim 1;

(b) amino acid sequences set forth in SEQ ID NO:235, 237, and 245;

(c) sequences having at least 70% identity to a sequence encoded by a polynucleotide of claim 1; and

(d) sequences having at least 90% identity to a sequence encoded by a polynucleotide of claim 1.

3. An expression vector comprising a polynucleotide of claim 1 operably linked to an expression control sequence.

4. A host cell transformed or transfected with an expression vector according to claim 3.

5. An isolated antibody, or antigen-binding fragment thereof, that specifically binds to a polypeptide of claim 2.

6. A method for detecting the presence of a cancer in a patient, comprising the steps of:

(a) obtaining a biological sample from the patient;

(b) contacting the biological sample with a binding agent that binds to a polypeptide of claim 2;

(c) detecting in the sample an amount of polypeptide that binds to the binding agent; and

(d) comparing the amount of polypeptide to a predetermined cut-off value and therefrom determining the presence of a cancer in the patient.

7. A fusion protein comprising at least one polypeptide according to claim 2.

8. An oligonucleotide that hybridizes to a sequence recited in SEQ ID NO:1-234, 236, and 244 under moderately stringent conditions.

9. A method for stimulating and/or expanding T cells specific for a tumor protein, comprising contacting T cells with at least one component selected from the group consisting of:

(a) polypeptides according to claim 2;

(b) polynucleotides according to claim 1; and

(c) antigen-presenting cells that express a polypeptide according to claim 1,

under conditions and for a time sufficient to permit the stimulation and/or expansion of T cells.

10. An isolated T cell population, comprising T cells prepared according to the method of claim 9.

11. A composition comprising a first component selected from the group consisting of physiologically acceptable carriers and immunostimulants, and a second component selected from the group consisting of:

(a) polypeptides according to claim 2;

(b) polynucleotides according to claim 1;

(c) antibodies according to claim 5;

(d) fusion proteins according to claim 7;

(e) T cell populations according to claim 10; and

(f) antigen presenting cells that express a polypeptide according to claim 2.

12. A method for stimulating an immune response in a patient, comprising administering to the patient a composition of claim 11.

13. A method for the treatment of a cancer in a patient, comprising administering to the patient a composition of claim 11.

14. A method for determining the presence of a cancer in a patient, comprising the steps of:

(a) obtaining a biological sample from the patient;

(b) contacting the biological sample with an oligonucleotide according to claim 8;

(c) detecting in the sample an amount of a polynucleotide that hybridizes to the oligonucleotide; and

(d) compare the amount of polynucleotide that hybridizes to the oligonucleotide to a predetermined cut-off value, and therefrom determining the presence of the cancer in the patient.

15. A diagnostic kit comprising at least one oligonucleotide according to claim 8.

16. A diagnostic kit comprising at least one antibody according to claim 5 and a detection reagent, wherein the detection reagent comprises a reporter group.

17. A method for inhibiting the development of a cancer in a patient, comprising the steps of:

(a) incubating CD4+ and/or CD8+ T cells isolated from a patient with at least one component selected from the group consisting of: (i) polypeptides according to claim 2; (ii) polynucleotides according to claim 1; and (iii) antigen presenting cells that express a polypeptide of claim 2, such that T cell proliferate;

(b) administering to the patient an effective amount of the proliferated T cells, and thereby inhibiting the development of a cancer in the patient.

SEQUENCE LISTING

<110> Corixa Corporation
Jiang, Yuqiu
Hepler, William T.
Clapper, Jonathan
Wang, Aijun
Secrist, Heather

<120> COMPOSITIONS AND METHODS FOR THE THERAPY
AND DIAGNOSIS OF COLON CANCER

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ataagccttg tgttgcagtc aagtttttac cacattctat agaccttgct gtacctg 477

<210> 19
<211> 374
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(374)
<223> n = A,T,C or G

<400> 19

agaaacttta gcattggccc agtagtggct tctagctcta aatgtttgcc ccgccatccc 60
tttccacagt atccttcttc cctcctcccc tgtctctggc tgtctcgagc agtctagaag 120
agtgcatctc cagcctatga aacagctggg tctttggcca taagaagtaa agatttgaag 180
acagaaggaa gaaactcagg agtaagcttc tagacccctt cagcttctac acccttctgc 240
cctctctcca ttgcctgcac cccaccccag ccactcaact cctgcttgtt tttcctttgg 300
ccataggaag gtttaccagt agaatccttg ctaggttgat gtgggccata cattccttta 360
ataaaccatt gngt 374

<210> 20
<211> 207
<212> DNA
<213> Homo sapien

<400> 20
acaagtgtgg cctcatcaag ccctgcccag ccaactactt tgcgtttaaa atctgcagtg 60
gggccgccaa cgtcgtgggc cctactatgt gctttgaaga ccgcatgatc atgagtcctg 120
tgaaaaacaa tgtgggcaga ggcctaaaca tcgccctggt gaatggaacc acgggagctg 180
tgctgggaca gaaggcattt gacatgt 207

<210> 21
<211> 557
<212> DNA
<213> Homo sapien

<400> 21
acaaagaatc cctagacgcc atactgagtt ttaagttcct taattcctaa tttaaggctt 60
ctagtgaagc ctcctcacag taggcttcac taggcccaca gtgcccctag acctctgaca 120
atcccaccct agacagactt tattgcaaaa tgcgcctgaa gaggcagatg attcccaaga 180
gaactcacca aatcaagaca aatgtcctag atctctagtg tggtagaact atgcacctaa 240
acattgctgc aaaatgaaca cactttttaga caccccctgca gatatctaag taagtggaga 300
agactatttt ttcaacaaac attttctctt tcaccctaac tcctaaacag cttactgggg 360
cttctgcaag acagaaagat cataattcag aaggtaacca tcgttataga cataaagttt 420
ctggtcaaaa gggttatagt taatgctctg cactttttcc tgcatcttat gcattacaat 480
gtctagtttg ccctctttcc ctgtgtttgt gtcataatag taaaaaatct cttctgttct 540
ggggtcatag cacctcg 557

<210> 22
<211> 541
<212> DNA
<213> Homo sapien

<400> 22
acctaggtgc tagtctcccc actaactgag ggaaaaaggt tcccaggtgg ggtcctctgc 60
ccactttgcc accacattca cattccaaat gggataatgc ctgaggggcc aagagtggtc 120
aggctgccct ggggtgaatg tcaccctgat gaggcccatc agctcttgcc cactcagtga 180
ggccagactt gtgctctaat ccactctcct gtgggtccct ggcctgtatg gcttatactg 240
gggagctggg cctctgggct gtccaaaccc aagggtcaca ctttgctttt cctttgttgt 300
ccccatttc catccttgct ctaagacaaa acttttccca gagaagaact ctttgttgtc 360
cccgctcagc tgtaattctg ccttttctac cttcattcca tccttcctct gcccagataa 420
agtccagcag aaattcctcc tttctacctc tctgggactc tgagacagga aatcttcaag 480
gaggagtttt tccctcccca ctattcttat tctcaacccc cagaggaacc aaggctgctg 540
t 541

<210> 23
<211> 486
<212> DNA
<213> Homo sapien

<400> 23
acaaaattgt tggaatttag ctaatagaaa aacatagtaa atatttacaa aaacgttgat 60
aacattactc aagtcacaca catataacaa tgtagacagg tcttaacaaa gtttacaaat 120
tgaaattatg gagatttccc aaaatgaatc taatagctca ttgctgagca tggttatcaa 180
tataacattt aagatcttgg atcaaatgtt gtccccgagt cttctacaat ccagtcctct 240
tagaaattgg tttctctctt tgggagattc agactcagag gcagccagag gggacaggtc 300
aagagctgaa ataatcacat aactactcta attttcttca ttctattgac tgtgtcaagt 360
tatagacaca gccaaagtgt ttttcttcgg cctctgatga tttgagaaga tgaagaacat 420
gagcaatttc tcattgctta aagaaaaact tggcacataa gaggctgagt gtagtagagt 480
atctgt 486

<210> 24
<211> 450
<212> DNA
<213> Homo sapien

<400> 24
actgatacat gctataacag agatgaactt cgaaaacatg ctaagtgaaa gaagccaaat 60
ccaaaaacaa taaaaacaca tattgtatcc tcaccctttt cgcattttag tgagcaatca 120
ttgcatatga atgtttatgg gaaaaatcaa tgtgtgctaa atcattgtat tccagtaaat 180
agattggact taaaacttga tacagaagtt gcaaataagt gggattgagt ttgattatta 240
tatagaaaat aattacatga ttcatttaag aataataata tccaccattt attgagcact 300
tactatgagc ctgtgtgcca aacatttcat gcatttctca tttaattctc acaataatcc 360
tgtgaggtag aagctattag gttgaatcat atgaacttgc caatatatga taatttctaa 420
gagttgggaa tttttgagga tgtgaatggt 450

<210> 25
<211> 638
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(638)
<223> n = A,T,C or G

<400> 25
gcaggtacac gtagcgcttc cccgacgtct tgtggatgat gttcttgncg taatagtagc 60
gtaagccccg gctcagcttc tcgtagttca tcttgggctt atttttcctc tttccccacc 120
ggcgggccac ctcatcgggg tcggcgagct taaactccca tccgtctcca gtccagctga 180
tgaatgactg gcaggatttg tctgatagca gctccaggag aaactgccac agctgaatag 240
gtccacttcc tgtgaagccg gccagcacag ctgcaggtat aactggtttg ccttgctcca 300
ccgggtcact cctctcttgg atgtaatcct tgaaagacat ggttggctta ttgaggcaga 360
gagactggct gcagtcatct tcgaagctct cgaaggaagg aacccgttgc acatccagca 420
aggacgactg gctgttccag gactggagga gggagtctga gctctcgaag ctgtccgcac 480
cgttctcagg ggagtcgtgg tctttgggcg tcccagaatt gttggtgagc aaattcaagt 540
tgctgcctgg gaagtcctga ctgacagagc agtaggtgac gctgacggag ctgagccgag 600
acttggggaa catctgaaac tnctgctcaa agctgagt 638

<210> 26
<211> 469
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(469)
<223> n = A,T,C or G

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<400> 26
naggtaccaa atggagaaaa ctctttccgg agacgttcat catcaatacc atcatcaaga      60
tttttcacat aaagattaac accctggtat ctggtgatcc tatcttgttt catctgttca     120
aatttgcgct taagttccgt ctgccgttcc accttttct gagctcgacc aacataaatt      180
tgttttccat tgagctcctt tccgttcatc tcatccacag ctttctgtgc atcttcatgc     240
ctttcaaagc ttacaaatcc aaatcctttg gattttccac tttcatcagt cattactttc     300
acacttaagg caggcccaaa cttgccaaag agatccttaa ggcgctcatc atccatgtct     360
tctccaaaat tcttgatgta aacattggtg aattcttttg cctagctcca agttcagctt     420
ctcgtcttta cgagacttaa atcggccaac aaatactttg cgatcattt                  469

<210> 27
<211> 364
<212> DNA
<213> Homo sapien

<400> 27
actctgctat ggtgctggct tcctttaaac tcaggataga tgccaggtgg gctccgtttc      60
cgtaagactg acactcgagc tcggcatcag accagttcct cagcttcctg aagtaaccat     120
agcaattgga cttgtggtaa aaccatccag gagcacagct gggtctcatg atgatatcac     180
ccaggactcc tgttttggcc aggcagctca gcaataggag cagccgcatg cttctggaag     240
ccatcttcct cctaccctga ggatgtagct agtgcaagga tctcagagac cttactagcg     300
cttctttgaa actcctgggt tctccttgat ctgcaaatct gtttggcaac caagactcta     360
aggg                                                                   364

<210> 28
<211> 714
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(714)
<223> n = A,T,C or G

<400> 28
ccttcgagaa gatccctagt gagactttga accgtatcct gggcgaccca gaagccctga      60
gagacctgct gaacaaccac atcttgaagt cagctatgtg tgctgaagcc atcgttgcgg     120
ggctgtctgt agagaccctg gagggcacga cactggaggt gggctgcagc ggggacatgc     180
tcactatcaa cgggaaggcg atcatctcca ataaagacat cctagccacc aacggggtga     240
tccactacat tgatgagcta ctcatcccag actcagccaa gacactattt gaattggctg     300
cagagtctga tgtgtccaca gccattgacc ttttcagaca agccggcctc ggcaatcatc     360
tctctggaag tgagcggttg accctcctgg ctcccctgaa ttctgtattc aaagatggaa     420
cccctccaat tgatgcccat acaaggaatt tgcttcggaa ccacataatt aaagaccagc     480
tggcctctaa gtatctgtac catggacaga ccctggaaac tctgggcggc aaaaaactga     540
gagtttttgt ttatcgtaat agcctctgca ttgagaacag ctgcatcgcg gcccacgaca     600
agaggggggag gtacgggacc ctgttcacga tggaccgggt gctgaccccc ccaatggggg     660
actgtcattg gatgtcctga agggagacaa tcgctttnca tgctggtagc tggc            714

<210> 29
<211> 373
<212> DNA
<213> Homo sapien

<400> 29
acttgagatc cacagtcacg tgaactttgc cggtctcttt acatctgccc acttcatttt      60
cattctttcc ttcccacaca atggtttttc caatgtgcaa gaatgatttc tcgacaaatt     120
cccggacact atggacctcc ccagtagcta taacgaaagc cttccggtca tcattctgca     180
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acatcaacca catagcctcc acatagtcct tggcatggcc ccaatctcgt ttggcatcca 240
gatttcccaa actgaaacat tccagttgtc caaggtaaat cttagctact gaccggctaa 300
tttttcgagt aacgaaatta gcttctcttc tgggactctc atgattgaag agaatgccgt 360
cactgcaaag aga 373

<210> 30
<211> 485
<212> DNA
<213> Homo sapien

<400> 30
aaaactacga ctcagcatac attttcccac atacattttt acattgtacc ttaggactca 60
gtcatctcca cttaaattga tgacacaagc agctaataac catttctggg tttctgccta 120
accccctaat tgtctgttaa agccaattct ctgggtgtcc cagtgagtgg tggctttttt 180
tctttccaca ttggcacatt cacttctccc actcttggca tgtaagaaat aagcatttac 240
ataattggaa aaatctggat ttctgatgcc aaagggttaa agcttcttgg atttcatttc 300
attgatatac agccactatt ttatttttga tcagtggcct ttgggccact gttcagggta 360
ctgaccatca gtgtcagcat tagggttttg gtttttgttt cttttgggtc tttcttttttt 420
ggcacatgtg aatcttgttt tgtgtaaaat gaaattactt tctcttgttc tctgatgatg 480
ggttt 485

<210> 31
<211> 342
<212> DNA
<213> Homo sapien

<400> 31
acacattaag catccccagt tcccctcgca cacccctttt cccagccact agtaaccatc 60
cttctactct ctatatccat gagttcaatt gttttgactt ttagatcccg caaataattg 120
agaacatgca atgtttgtct gtttctggct tatgtcactt aatatagtga cctctagttc 180
catccatgac tccttaactg cccctgaatt tttgacacta ttatttttaa gtattttgga 240
aaactcacac ctgttctcat ttttaaacct taataataac aatttcctac taagctaata 300
aaacttcccc ttatattatt tgtaatgtgt gcataacata gt 342

<210> 32
<211> 331
<212> DNA
<213> Homo sapien

<400> 32
acagtatgtg gcatttccag gtatgactga gtgtgagaga catgtcagag gctcttcagt 60
gatttcttgc tattgaccga tgcttcactg tgccaaaaga gaaaaaaaat gttgggtttt 120
gtaattaaat tatttatata tttttgaaac ccgaattgaa aatgttgcag gcaacgggct 180
acagctttat tagtggttct ctaactgtgg tctccttggg ccaagcaatt tctttaaagg 240
aaaagttgat tatgtatgtg gagtgccagg accactgcct tgaaagcaag tgtgattttt 300
atttttaata ttattttatt tgtgtctgtg t 331

<210> 33
<211> 381
<212> DNA
<213> Homo sapien

<400> 33
acactgttgg tgttatatgg ggatggggtt ctcggtaatt ttgtttatta tttatgttta 60
ttattatgtt ttatcattaa ttattcaata aatttttatt taaaaagtca ccctacttag 120
aaatcttctg tgggggtggg agggacaaaa gattacaaac caaaactcag gagatggtaa 180
cactggaatt gataaaatca cctgggatta gttgtataac tctgaaccac caaacctctg 240
ttatcaagcc ttgctacagt catggctgtc cagaaagatt tacagttatt tttctgagaa 300

aggatccatg ggctttaaga acttcagaac tttaagaact tcagaagttc ttaagttgct 360
gaagctcaag taacgaagtt g 381

<210> 34
<211> 315
<212> DNA
<213> Homo sapien

<400> 34
acgaaactgt atgattaagc aacacaagac accttttgta tttaaaacct tgatttaaaa 60
tatcacccct tgaggctttt ttttagtaaa tccttattta tatatcagtt ataattattc 120
cactcaatat gtgatttttg tgaagttacc tcttacattt tcccagtaat ttgtggagga 180
ctttgaataa tggaatctat attggaatct gtatcagaaa gattctagct attattttct 240
ttaaagaatg ctgggtgttg catttctgga ccctccactt caatctgaga agacaatatg 300
tttctaaaaa ttggt 315

<210> 35
<211> 567
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(567)
<223> n = A,T,C or G

<400> 35
tacttcttaa aanacatata acacaatgtg gtagtagtag gtgtaaggaa ggtaagtttt 60
ttcatagtgg tatgcaaaca tatcattgaa atattacata gatataaaga cttagggaat 120
aaaaatagca gcaacaaata cttgatagat ttatcctact tgggagaaat attttgtagc 180
agagtattta gtatacttag aagttgattt agcaattagg ctttaatgac cttacaaagt 240
gaacataact gaacacaagt atttttttcaa tgcaagatga ggatgaaaat tttacatttc 300
aacccatctg gctaaagtta agacttagca aaaattaaaa tgttgccttt gtccaagtat 360
agattaaggc aacaaacata tttgggtgtg taatttgaag ttttggactg aaatatcttt 420
gcaagtatcc acataaaatt ctgtaatgcc ttataattat attctaataa ttatgcatta 480
tactaagaca ccattaagaa cagttgangc actacactaa atcaaaccat aaatgaggaa 540
aaaactttta atggtctttt ctagaag 567

<210> 36
<211> 265
<212> DNA
<213> Homo sapien

<400> 36
acaagtggtg gccacagaag taggggggtc ttccttaagc tctgtgtcag agttccacct 60
gatccttatg gatgtgaatg acaaccctcc caggctagcc aaggactaca cgggcttgtt 120
cttctgccat cccctcagtg cacctggaag tctcattttc gaggctactg atgatgatca 180
gcacttattt cggggtcccc attttacatt ttccctcggc agtggaagct tacaaaacga 240
ctgggaagtt tccaaaatca atggt 265

<210> 37
<211> 476
<212> DNA
<213> Homo sapien

<400> 37
actgtatgtg ttttgttaat tctataaagg tatctgttag atattaaagg tgagaattag 60
ggcaggttaa tcaaaaatgg ggaaggggaa atggtaacca aaaagtaacc ccatggtaag 120

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gtttatatga gtatatgtga atatagagct aggaaaaaaa gccccccaa atacctttt     180
aacccctctg attggctatt attactatat ttattattat ttattgaaac cttagggaag   240
attgaagatt catcccatac ttctatatac catgcttaaa aatcacgtca ttctttaaac   300
aaaaatactc aagatcattt atatttattt ggagagaaaa ctgtcctaat ttagaatttc   360
cctcaaatct gagggacttt taagaaatgc taacagattt ttctggagga aatttagaca   420
aaacaatgtc atttagtaga atatttcagt atttaagtgg aatttcagta tactgt       476

<210> 38
<211> 424
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(424)
<223> n = A,T,C or G

<400> 38
tacaagaacc tcactcactg gacattgann ttctactgtc caatcccaac tnactgctgt    60
tnantggaaa cctgattctg gcagctcatt tatcttggtt tcctcatttg taaggtcgtt   120
cagttggact gatcatctct gagggccttg aagccctaac aagtctatca tgatcccaga   180
tgtaaaatat atatatgtgt atatatataa tttcagctga gaagtgagtc ttcacaccaa   240
gtctactttt tgcaagttac tgggtttctg tcttcaccat cttctgaaaa gtctgcttct   300
gttggttcag tttctggggt catctgagta gagagattct gaaacagaca ctgatgttaa   360
tttgggggac tacttttctc atgcaaacag gggagctcct ancaatcctg agaggngctg   420
catc                                                                424

<210> 39
<211> 493
<212> DNA
<213> Homo sapien

<400> 39
acattgtagc cctctgcctc tctaccctta acagctgcat cgaccccttt gtctattact    60
ttgtttcaca tgatttcagg gatcatgcaa agaacgctct cctttgccga agtgtccgca   120
ctgtaaagca gatgcaagta tccctcacct caaagaaaca ctccaggaaa tccagctctt   180
actcttcaag ttcaaccact gttaagacct cctattgagt tttccaggtc ctcagatggg   240
aattgcacag taggatgtgg aacctgttta atgttatgag gacgtgtctg ttatttccta   300
atcaaaaagg tctcaccaca taccatgtgg atgcagcacc tctcaggatt gctaggagct   360
cccctgtttg catgagaaaa gtagtccccc aaattaacat cagtgtctgt ttcagaatct   420
ctctactcag atgaccccag aaactgaacc aacagaaagc agacttttca gaagatggtg   480
aagacagaaa ccc                                                      493

<210> 40
<211> 464
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(464)
<223> n = A,T,C or G

<400> 40
acaaaacaca caaacatcac tttacttgga aaattatttt catcatactg taaacatctc    60
ttcccctaca tctggacatt ttgaaatagt ctttggtatt actagttatt gtgctttgaa   120
acagaaactt gcagaatttc tgtagtagtg ctacataaag atataaataa gaaaaatgca   180
cttggaataa gttacattta gctgcttttg cataattttc aaaaactaca gtgtatgcct   240
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agtcacagtt ttatgagaaa gaatatttcc tttttcaact taattttaag gaacacttaa    300
tcattttggc taagtatcca tttttggagt ggatctgatg agttgcatga cactaaactt    360
ggatgctctc catttgctga aaggcacatt tttaagaatg gattgnatag aagttgatcc    420
ttctggatct cccatatctg ctctccagtg acaactgnct tgtg                     464

<210> 41
<211> 557
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(557)
<223> n = A,T,C or G

<400> 41
acagtgatag gtatctttct ttggagtttt ttttttgngc atatgtgtat agttttatgg     60
gttctgagtt ggtgaccana aagttgcatg tagngctggc acttacttaa taactattca    120
tgatattgtt aataacttgt tataggattg tattcccaat tacagtctct aanattgtaa    180
ttgatattat ctganaggna gngngacaac tttcttttgt tgttacatta agccgaaaac    240
ataatactaa tagacaacta acagtttgct tatcaggcac atcaactaag gcacctcccc    300
ccatgctaag tttctcctgg atatatggaa gttgattgtt tcccagttna aaaacttgaa    360
ctaatatctc ctaaaaaaat ctgagtccat attgttttta ttttacttag ctanaatctc    420
atagcangtt aaagtcatat ccttatcccc actaaaaata actatgtnta tgtgagagga    480
atatagtatg tgggagctgt attaaatact attacaggtg ttacagaatc tttaaataaa    540
tggacatgga ccaactt                                                   557

<210> 42
<211> 255
<212> DNA
<213> Homo sapien

<400> 42
actatcaggc tttgtgctga tttcctgaac aaactgcatt atattatgaa aacaaaagga     60
aaagaagaaa taataaaaac tatactccca tatttcactt acagtgtttg agttcctgga    120
aggacctata taatggaggc agcattcaaa caagaaatta tgccaatcaa ctgtcaaatt    180
ttcactataa ttttcctaaa aaggcgtttt tcccccaata tctattaatc tcaaagaaac    240
ataagttgtg aatgc                                                     255

<210> 43
<211> 349
<212> DNA
<213> Homo sapien

<400> 43
actccagcag atttaatatt ggcatccatc atctagtcaa acctctcaca tgttcttcaa     60
atcaatcaaa tttgggattc tcaacatttt ctgtgtcaat aaaaggtgtg gaattagtag    120
attcgatgaa gacctgtttt tccttgccac attggacttc cagacgccat ttggattggg    180
tttagaagat ggggaaattt agaagacgtt tcttggcctg agtctcttaa gagtagagat    240
gcagaagaga gagtgagacc acgaagagac tggctgttga ctgcagggca ccaccagccg    300
ccttggtggt ggcattagtt ggatttgggg ccaacccaga gttggaagt                349

<210> 44
<211> 483
<212> DNA
<213> Homo sapien

<400> 44
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accaaaccat tttatgagtt ttctgttagc ttgctttaaa aattattact gtaagaaata    60
gttttataaa aaattatatt tttattcagt aatttaattt tgtaaatgcc aaatgaaaaa   120
cgtttttttgc tgctatggtc ttagcctgta gacatgctgc tagtatcaga ggggcagtag  180
agcttggaca gaaagaaaag aaacttggtg ttaggtaatt gactatgcac tagtatttca   240
gactttttaa ttttatatat atacattttt tttccttctg caatacattt gaaaacttgt   300
ttgggagact ctgcattttt tattgtggtt tttttgttat tgttggttta tacaagcatg   360
cgttgcactt cttttttggg agatgtgtgt tgttgatgtt ctatgttttg ttttgagtgt   420
agcctgactg ttttataatt tgggagttct gcatttgatc cgcatcccct gtggtttcta   480
agt                                                                 483
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<210> 45
<211> 281
<212> DNA
<213> Homo sapien

<400> 45

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acatcgagaa tccacgcccg gggaccagta ggacttgagg gactgcttac tactaagtgg    60
ctgctgcgag ggaaggacca cgtggtctca gatttctcag agcatggaag tttaaaatat   120
cttcatgaga acctccctat tcctcagaga aacaccaact gaaaagagcc aggaaaaccc   180
gggaattttc caaaaggtct tcacgttaaa cttgtcttat ctcaggagag agcccgctct   240
tgtctcccag ttcctggtag ggtctgcctg ttggaaagtg t                       281
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<210> 46
<211> 587
<212> DNA
<213> Homo sapien

<400> 46

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acagcccggc ctcccttgat gcatttggcg cgttcctgaa aagttgtgtg taaaggaaga    60
atttgccatc aagccatttc cccttttgt ttctaaaatt atttcagaga tgtgtgctcc   120
tggagggaaa aagaaatacg gcctcaacag attaaaaaac aaaagtcaca cttaaggatc   180
cttctagtca catcagcagt gttctgcctt tatgtagtag ttgggcatat aatccttcca   240
cacagcccct gcagggaaag gctaatctta cggataatcc acgtgagatt tccacacaag   300
agaaaagcac acgcatagtg aaatgtcagt cttttcagta atgaggatac ctttaaggca   360
ctcttggact ctcggcaacc acaacataat agttgaaaga tcaagattgg ctccacgaaa   420
gtgatacgga ggttaggatg ctacttgctg caaacaagcc ctactttggc caacatcctg   480
cttatttctc aaaaaagagg gacagtgaaa acaaaaacga cattgggaca tgctgctcaa   540
ggtagttata tatacgataa gttgtatata tgatcactgg tagccta                 587
```

<210> 47
<211> 317
<212> DNA
<213> Homo sapien

<400> 47

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gaggactctg acagccataa caggagtgcc acttcatggt gcgaagtgaa cactgtagtc    60
ttgtcgtttt cccaaagaga actccgtatg ttctcttagg ttgagtaacc cactctgaat   120
tctggttaca tgtgtttttc tctccctcct taaataaaga gaggggttaa acatgccctc   180
taaaagtagg tggttttgaa gagaataaat tcatcagata acctcaagtc acatgagaat   240
cttagtccat ttacattgcc ttggctagta aaagccatct atgtatatgt cttacctcat   300
ctcctaaaag gcagagt                                                  317
```

<210> 48
<211> 512
<212> DNA
<213> Homo sapien

<400> 48

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acacttgtat ggcttttcac cagtgtgagt cctcaggtga gcttttaaat gagaagactt      60
ggtataaact tttgtgcaac cagggtaatc gcagtagtgg atgcgtcgtt tctccaaatc     120
ggggttactc cttctattgt atctgacagg ttggatgttt tgtgagttaa ctggcagggt     180
ggtgggtaaa tttggattgt gaattgccag tttagaagca attgtagcag cataggatgg     240
aggtggggtt aaattctgga gcatctctgc ttgtctatct ggacttccag gctctgagct     300
tggtggtgac gggggaaagt aagtggcctg ttgtggaaga aactgacttg gcattgtgta     360
tgtgcaaggg ggcatgccct ggaattgttt cactgcagtc tgcggaacag cagaggtgtg     420
tgtgttaagg cctgccatgg cagctgacat agaaacatta agagtgtcca ttgctgctgt     480
ctgatttgta gaactgggca tatctagatc cg                                   512

<210> 49
<211> 454
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(454)
<223> n = A,T,C or G

<400> 49
acaggattca ctaactgttt cgaatgaagc ccaaactgcc aaggagttta ttaaaatcat      60
agagaatgca gaaaatgagt atcagacagc aattagtgaa aactatcaaa caatgtcaga     120
taccacattc aaggccttgc gccggcagct tccagttacc cgcaccaaaa tcgactggaa     180
caagatactc agctacaaga ttggcaaaga aatgcagaat gcttaaaggc tgaatgtagg     240
attcttcagt atgtggaaag acaaggattc aacgtgtggt catatgataa ataagtgatt     300
tataaacaag agtgatattt tgctagggct ttcaaagtta accggttttc tagcctcatg     360
gaatactgtt gaacctatag cgttgtcttg attcttttgt gttctctgcc ttgtaatttt     420
ctgttactgc tatatctacg tgtaaatctt tntt                                 454

<210> 50
<211> 374
<212> DNA
<213> Homo sapien

<400> 50
actatcccat gttgcgcagt aatagatggc ctcgtcccca gtccggagtc cggtgatggc      60
cagggcggct gacgtgccag acttggtggc agagaatcgg tcaggaattt ctgagggacg     120
gccatcattg tgataaatga ggagtttggg ggctgttcct gagaattgta gataccacga     180
cacataatta gttccaatgt tggaggcgct tccagagcag gacatggaga ccttctgtcc     240
tggggccgca gagactgagg gcggctgcgt caagatggac tgggcccagg accctgtgca     300
gtgaatgaga agggtgagga ggagagggga gcaggtcatg atgaagattg tcccgagtcc     360
tgccttctgc gctc                                                       374

<210> 51
<211> 250
<212> DNA
<213> Homo sapien

<400> 51
accagatatt ttctatactg caggatttct gatgacattg aaagacttta aacagcctta      60
gtaaattatc tttctaatgc tctgtgaggc caaacattta tgttcagatt gaaatttaaa     120
ttaatatcat tcaaaaggaa acaaaaaatg ttgagtttta aaaatcagga ttgacttttt     180
tctccaaaac catacattta tggcaaatt gtgttcttta tcacttccga gcaaatactc      240
agatttaaaa                                                            250

<210> 52
<211> 351
```

<212> DNA
<213> Homo sapien

<400> 52
acgaaagggt ttgtaccaat attcactacg tattatgcag tatttatatc ttttgtatgt 60
aaaactttaa ctgatttctg tcattcatca atgagtagaa gtaaatacat tatagttgat 120
tttgctaaat cttaatttaa aagcctcatt ttcctagaaa tctaattatt cagttattca 180
tgacaatatt tttttaaaag taagaaattc tgagttgtct tcttggagct gtaggtcttg 240
aagcagcaac gtctttcagg ggttggagac agaaacccat tctccaatct cagtagtttt 300
ttcgaaaggc tgtgatcatt tattgatcgt gatatgactt gttactaggg t 351

<210> 53
<211> 546
<212> DNA
<213> Homo sapien

<400> 53
acatggacat tctgcaaacc cagctgtcac atttttcttg caactccttt tgcaaaagca 60
gactaaaatg ttttaaaatg tgaaaaaaca ttattttttc aaagcaagaa aataatttac 120
tgccctctta cataatgtat ttataaagtt tttccagata aactaatcaa ataaattaga 180
ataatgtgac aacattacaa atttaatttg ttagctgcat tccttctgat gttaccacga 240
tagaatgtta ctgatgattc agggctattt ctgaagtctg tatgttgctg ctgtccccag 300
tgatggtgga cttatctttg ccttacctga tcacaaatta tgttggggaa aataaagatt 360
taatatttct ttaaatagaa aaagaatttg gttttgctcg tttaagagca atgagaaaat 420
gatggaatgt tgactgtgtt tggcacacag gacacggacc ttcatggaag tccttgctct 480
gcgtggcatc tgtcagcttt tcacctttca ttcttattct tcacttttgc tgctgagcct 540
agctgt 546

<210> 54
<211> 631
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(631)
<223> n = A,T,C or G

<400> 54
acngttttaa ccaatacnna naagcantaa agcaataata tctgaagcat tatttaagaa 60
atctcaatac acgatctctg aagttcctaa aattctggca ctaattctaa tgtgaactta 120
gtagcaaaag acccagaaat agtaagccct tgacctaaaa actaactgat ttgtatgata 180
ttcatgcaga aacaatgatg aaatggagtc aagttttcta gtgtcattgt tatcaaaata 240
actgtcaaaa tagtaagttt gaaacttaaa tgagcacaaa ataaaatttt gttttctaac 300
aagaccagat ttctttttaa aaataattct gagttagaca aagtgatttt cctaaaagct 360
agctgaagct accttaaata tcccctattt taagttacag catctctaaa taagttaatc 420
acacaagata gtttaaatac acctttaggt gtaggggagg ggagaagcgc ctctttttct 480
aatgcagctg ttttaatttg aagcttttgc acaaaatcag atagaaacat taatgcctaa 540
ctcataatga cccttgatta cttgtaattt tggactagaa ataatgtggc tttgaacatg 600
ccagtgttag accatactga cttaaaaaaa t 631

<210> 55
<211> 408
<212> DNA
<213> Homo sapien

<400> 55
accaatatat ccccagaaag aattgcaatt taccaaggtt ttcacgtgtt ttgagagaaa 60

```
tcttactgaa agactagtga tgtccatttt ccagtaaata ctgagcgaaa aacaattttt     120
ataccccaat ctgaggtata aacttgcttt ttgtgggatc acaactgctg taaattagac     180
aattgtagca acaatccaag acaataacag aatgcctatg acagtctgcc atattctggt     240
gagtgtctat caaagctcat catgattttt tgtgagatct tccccgtaat tggtagcttg     300
gcttccaaca aacatgttcc agttctccaa tatttcctct ttagttagct tctcatcctt     360
gtttttgtct gattcatata ccagatgcct ggcctcagcc tgtgcgtg                 408

<210> 56
<211> 567
<212> DNA
<213> Homo sapien

<400> 56
actgtgggtc gaagtaatgg atacggacgt aaccatcttc gccgccgctg ctgtagctct      60
tgccatcagg atggaaggca acactgttga taggtccaaa gtgacccttg actcttccaa     120
actcttcttc aaaggccaaa tggaagaacc tggcctcaaa cttgccaatc ctggtggagg     180
ttgtggttac atccatggct tcctgaccac cgcccaggac cacatggtca tagttggggg     240
agagggcagc tgagttgaca ggacgttctg tccggaaagt cttctgatgt tcaagagttg     300
tggagtcaaa aagcttggct gtgttgtcct tggacgcggt cacaaacatg gtcatgtccc     360
tggataactg gatgtcgttg atctgccggg agtgctcctt aacattcacc aacacctctc     420
cagacttggc actatactgg ttgagctctc cactctcatg gccagcgatg atgcactccc     480
ccaggggtcc ccaaacagca ctggtgattt tagagtcatt gcaagggatc ttcatgtagg     540
gctcattggt gtcaatctgg ctcggat                                         567

<210> 57
<211> 411
<212> DNA
<213> Homo sapien

<400> 57
acccttcctt gtccgaagga gctgaccagt attgatgaga gagtccaggc agctcctgaa      60
gttcagctgg tagtttgttc tctgaacatt tggtctcttg aaggcacagt atatctgggg     120
cttcttcctt tacccaatct aatcctttct tcttaatcca ggctcgaagc ccatccacat     180
tccaagagca gatcttgagt gtggcaggtt tgccactggg tgaggttttc tgatctgggg     240
ggtcctcata cagggctggg ccctctcctg ctgcctcttt gtcatttttc tttgcggccg     300
tcttactctt cttggcctct ggctctgtcc tgagctcatc cccgtcttcc gccaccgctc     360
cctttttccc acgcttcggc attcccgtta cgaacgccct tgggcagctg t              411

<210> 58
<211> 589
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(589)
<223> n = A,T,C or G

<400> 58
acattaatac aaacatactt gcagtctgag cgaagatggg aatggaggct gaggaggtca      60
aaggacgaaa ggtcagccct aaagacaggg tgttttgtta ttatggtaat tacaccttca     120
taccttctat aatattcatt gacagacggt gacatcaaca ggtgtagttt atcatgttct     180
gtgtagagaa ctaaactacc ctactgtatt tgccatgccc ccaattccaa gaaaacggca     240
aaaaattagc ccatcccatt cctcatcaca aagatcttaa ctgcacccct gcaacacaag     300
acttttccaa taggacaaaa cttcaaacag cattgtatac caaatgattg cggatcaaaa     360
ttaaatttac aggaacacaa tactgaagca ctccactgtt gctgtaaaaa ctgctggaaa     420
cagaatctgt caactggcca aattttatcc ttaattatta tccaaacagc cgtcctcttc     480
acatctatcc ggatgatgct aatctactac cctgtccact aggttagcaa gttgtaggaa     540
```

caactcttca ccatttctcc caccctaaga ggtacctgcc cnggcggnc 589

<210> 59
<211> 440
<212> DNA
<213> Homo sapien

<400> 59
acatgaggca gttgagcagc actggagaac cttcacggtc cacacggaac tccccagttg 60
gagtataata gtcattctcc ttgatatgtt tgcctgtatc tgtgctccct ccaatccgga 120
ccatccaaag aaacttgttg atatcatcag aggaataccc agtgaggcct ccaaaaatga 180
ccagcacata gctgacatcg agctccctca tgatctcata ggctttttcc tctgtggacg 240
ccattgcctg ccctactcga gaaatatggg tattattcca tgtgttattg tccactaaaa 300
ttgttcggtt tgccatagct gtaatctgat agccataatc ccaccaggac atgaccttcg 360
catcctctgg agtattatga cgaagccaat aatatgcttc tcggaagtca tcaaatatga 420
tcctactgcc atccccacca 440

<210> 60
<211> 417
<212> DNA
<213> Homo sapien

<400> 60
acctggaaga tcaagatcta cagctgccta tttccacatc tttcaatcca tctggctcct 60
taaatagggg aaaaagccct tatttggtgg agaagcattt ccaaaatgaa gttacaggtt 120
ctattaaaac ttactgtcac atcaactgtt aaaatagggc cttttgtgtt ttgttatttc 180
accttaatat caccagaatt cctgtaattc cacaattgtg attttactat gtagaagata 240
attcagttct agtctattgc tttagatgta aaaacagctg aaaacccaaa gtggattaga 300
attgctgaag gatttccctg ccgttgtttg atacaatcta ttctcttgat tcttgatagg 360
tgcatagaaa gcctaactta aaattctttc tacaggaaca tgtctgattt caggagt 417

<210> 61
<211> 354
<212> DNA
<213> Homo sapien

<400> 61
acctcctgtg ttgcagagtt tctttatcca catccaccca accagcagca tcagccacag 60
gactggtctt gaggacatct ggtgggctca ttggaggtgt gacatgaagg atttcatatg 120
aaatcacttg ggtctctcct ggtttgtcca ggttctcaaa tacagcctct tgtttatcgg 180
ctcggacttc aatgaggttt ttcttgtagt taacagtgag gttccgctcc tggatgatct 240
cctgcagggc atctgcatac ttcttaaccc cgaaaatggc tccaagagaa gtgttgaaaa 300
tgatattggc cttggatcgc ttccctgtct tcctgaagta ggcttctgat aagt 354

<210> 62
<211> 205
<212> DNA
<213> Homo sapien

<400> 62
accccttcc acttcgtctc ccctagctcc tagaagcaac cactgatgtg atttctacca 60
aatccagttt tggtcctact aaatatactc ttttgagact ggcctctttt actcaccata 120
atgcctttgt aattcatcca tgctgttgtg tgtatcagca gtttgttcct tttcattgct 180
gagtagtatt ctattgtaga gatgt 205

<210> 63
<211> 325
<212> DNA

<213> Homo sapien

<400> 63
acacacgggt tccggatcaa tgctcgggcc aacgccactg cctgtcgctg accccctgac 60
agctggctcc cagcctcgtc tacctctgtg tcatagccct gagggagtcc agagatgaaa 120
ctatgggccc cagactttac tgcagcagct gtgatttcct ccatagttgg cttctgggtc 180
aggccatagg caatattttc ttgaagactt cttccaaata cctgtggctc ttgtcccact 240
gcagccacct gcctgtgcag gtagcggtgc tcatattggg gaaggggctt cccatccaac 300
agcagctgtc cccggtggg ctggt 325

<210> 64
<211> 599
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(599)
<223> n = A,T,C or G

<400> 64
actttgatgt ttgaacaacc ttttcttgat cacttcttcg caataaaaat atgacatatg 60
tagtaaacct taaaaaattt cgtgtaactt tatggctcta cgctggaatt cttctgaagt 120
gagtaatcat cacaatcatc tttagtatat aatggatcaa aatgacacga ttgcaaatat 180
tgataacaca cagttataaa aggtgaaatt ctattgggaa cacatctctt agtgagatag 240
atggggctga cccaccaatt aattcattta tctggatgaa tagttcctac tggtagatta 300
acagggttca ttttcaattc tgttgttttc acagatacaa gtgctgagaa atggttttac 360
ataaataggt gagaatgcta gtagttttgt tgtaagcatg tcaatcaatc gtttggtttc 420
tttccgagtt gcatgccaaa aaccaaatag tgttccttca tcagctgaca attcatgggc 480
caccattaat tttgttgaaa gcaaagaact ggaaaccatc tgacttgaaa agaatttggt 540
atcctggtat tagaggcatt cactttctct agngactttt aattatacta attactctc 599

<210> 65
<211> 373
<212> DNA
<213> Homo sapien

<400> 65
acattaaagt gtgatacttg gttttgaaaa cattcaaaca gtctctgtgg aaatctgaga 60
gaaattggcg gagagctgcc gtggtgcatt cctcctgtag tgcttcaagc taatgcttca 120
tcctctctaa taacttttga tagacagggg ctagtcgcac agacctctgg gaagccctgg 180
aaaacgctga tgcttgtttg aagatctcaa gcgcagagtc tgcaagttca tcccctcttt 240
cctgaggtct gttggctgga ggctgcagaa cattggtgat gacatggacc acgccatttg 300
tggccatgat gtcaggctcg gcaacaggct ccttgttgac actcaccaca ttgtttttca 360
agctgacttc cag 373

<210> 66
<211> 520
<212> DNA
<213> Homo sapien

<400> 66
acgtgagcca gtcatccata cactaaggcc tagttgagaa aaacctttga ttcaggatgg 60
ctgggttact aaccttgaaa tgtaagagat ctggttttga atgtaaaagt tgcaacacac 120
aaacggaagt cttaaaaact ttttgctctg gtcagttaca ggtggatccc caataatctg 180
tttttggttt tctgatggaa ataatagaat taggggaaat caaatctggt tggtaggtgt 240
ctacagtatt agaagagggt ataagggcac tgtttaacac taagttctaa tacttccaga 300
aactgtgcat tccagatcta catactaaat gctcttatca ttttgaaatg ggctcttgat 360

taatagaccc atatttttta gtggcttcta tgttgtatat ttgtctaaaa tgaaagctct 420
tttgcgttct aaaactacaa tatatgtcat cttattttcc ctgagtatcc aagtatagtg 480
cagattctat gtaaaactac taaatgacac tggaatatgt 520

<210> 67
<211> 241
<212> DNA
<213> Homo sapien

<400> 67
acagagatgg agaacgaatt tgtcctcatc aagaaggatg tggatgaagc ttacatgaac 60
aaggtagagc tggagtctcg cctggaaggg ctgaccgacg agatcaactt cctcaggcag 120
ctgtatgaag aggagatccg ggagctgcag tcccagatct cggacacatc tgtggtgctg 180
tccatggaca acagccgctc cctggacatg gacagcatca ttgctgaggt caaggcacag 240
t 241

<210> 68
<211> 487
<212> DNA
<213> Homo sapien

<400> 68
actttgaggg attggtggtc ttgggcccct cctggcccag gagatgtaga atacgggtgg 60
ccagcactgt gaactcgcag tcctcgatga actcgcacag atgtgacagc cctgtctcct 120
tgctctctga gttctcttca atgatgctga tgatgcagtc cacgatagcg cgcttatact 180
caaagccacc ctcttcccgc agcatggtga acaggaagtt cataaggacg gcgtgtttgc 240
gaggatattt ctgacacagg gcactgatgg cctggacaac caccaccttg aattcatccg 300
agatttctga catgaaggag gagatctgct tcatgaggcg gtcgatgctg ctctcgctgc 360
ccgtcttaag gagggtggtg atggccagcg tggcaatgct gcggtttgaa tctgtgacca 420
ggttctccag atccagatta caagctgtca cagctgacgg atgcttcatg gcaaccttat 480
tgagggt 487

<210> 69
<211> 415
<212> DNA
<213> Homo sapien

<400> 69
actagcttca agaagctttt ggtcagctac atttaaaggc acaatagggc ctttggattc 60
tttgtgtgta attggttttt cactgagtgg tttggaagta tctaaatcgg actttttact 120
atattccaca cttactacca catccttggt gccaggagat ttctcttgtg atgacaataa 180
ttcttcttgt ccttgaagat gagatatatc cagaccttct tttaggcgaa taaccactac 240
tccatattgt atgtcaaaag catcatgaaa taagtttata tacatatcca catccctcat 300
atctgcttgc aaccaatctt tcttaaatcc aaggacaagt gtgtttggct tcatacgacc 360
aagaccagca gcctgcatca aatactgtgc accttctctc aagtcatctg catgt 415

<210> 70
<211> 535
<212> DNA
<213> Homo sapien

<400> 70
acatcatgtc ttataaggaa gccattaagg tcactccact gccatgtatg caactgctgt 60
gtggctcgat atgatcaaca ctgcctgtgg actggacggt gcataggttt tggcaaccat 120
cactattaca tattcttctt gtttttcctt tccatggtat gtggctggat tatatatgga 180
tctttcatct atttgtccag tcattgtgcc acaacattca aagaagatgg attatggact 240
tacctcaatc agattgtggc ctgttcccct tgggttttat atatcttgat gctagcaact 300
ttccatttct catggtcaac atttttatta ttaaatcaac tctttcagat tgcctttctg 360

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ggcctgacct cccatgagag aatcagcctg cagaagcaga gcaagcatat gaaacagacg     420
ttgtccctca ggaagacacc atacaatctt ggattcatgc agaacctggc agatttcttt     480
cagtgtggct gctttggctt ggtgaagccc tgtgtggtag attggacatc acagt          535
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<210> 71
<211> 249
<212> DNA
<213> Homo sapien

<400> 71

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agcgggacga ggatgacgag gcctacggga agccagtcaa atacgacccc tcctttcgag      60
gccccatcaa gaacagaagc tgcacagatg tcatctgctg cgtcctcttc ctgctcttca     120
ttctaggtta catcgtggtg gggattgtgg cctggttgta tggagacccc cggcaagtcc     180
tctaccccag gaactctact ggggcctact gtggcatggg ggagaacaaa gataagccgt     240
atctcctgt                                                             249
```

<210> 72
<211> 297
<212> DNA
<213> Homo sapien

<400> 72

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acacactgat tgtgcggcca gacaacacct atgaggtgaa gattgacaac agccaggtgg      60
agtccggctc cttggaagac gattgggact tcctgccacc caagaagata aaggatcctg     120
atgcttcaaa accggaagac tgggatgagc gggccaagat cgatgatccc acagactcca     180
agcctgagga ctgggacaag cccgagcata tccccgaccc tgatgctaag aagcccgagg     240
actgggatga agagatggac ggagagtggg aacccccagt gattcagaac cctgagt        297
```

<210> 73
<211> 531
<212> DNA
<213> Homo sapien

<400> 73

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acttgtccca ctcctgttca gaggtcacat gcttatccaa aaactctgcc atcccaatgc      60
ccattctccg gcaaatgtcg gcaatcactg tttggtattt ctcagccaga tttctaaact     120
caagggagat cgttgggaag tcctccagca cctggcgatc cttctccttg ctctccatga     180
accgccagtc tggttggtaa aggaaagagt gaaagttgtg taacagcggg accttctttt     240
ccacactgat ggtcatgtca tcttccagtg tgtccagagc tcggagaacc agataaaata     300
tgcacactgc gttgcgcatt tccccatcca gcgcctggat aacagctgcg aaactgcgac     360
tggtctgatt gagatacttg tagcaagttt tcaggctgct gctgagcgag tcctggtcca     420
tcttgggcat caccttccgc ttgcccccga tccggaagcg caccaggttg tagaactctt     480
cggggtggcc aaggcatttc acgaactcca tcctggtgca ggcggcggac t              531
```

<210> 74
<211> 394
<212> DNA
<213> Homo sapien

<400> 74

```
actaaaactt acaataaata tcagagaagc cgttagtttt tacagcatcg tctgcttaaa      60
agctaagttg accaggtgca taatttccca tcagtctgtc cttgtagtag gcagggcaat     120
ttctgttttc atgatcggaa tactcaaata tatccaaaca tctttttaaa actttgattt     180
atagctccta gaaagttatg ttttttaata gtcactctac tctaatcagg cctagctttg     240
ctcattttgg agcctcacta aaataacaga tttcagtata gccaagttca tcagaaagac     300
tcaaatggaa tgatttacaa aatagaacac tttaaaccag gtcagtccta tctttttgta     360
gctgaaggct atcagtcata acacaatttc gcgt                                 394
```

<210> 75
<211> 369
<212> DNA
<213> Homo sapien

<400> 75
acattggtga tcggagtata gttggagcgc tttgtcatga tttccaggtt ggctttgtcc 60
acagctatgt tggccaatgc accttgagcc tcaaagctgg caaatcgtcc aaattcttca 120
agccgccaga ccgtctcctt ctttgccata tccacatgga aaatctcatc accatcaaag 180
tcaaacataa actcgcctga ttggtcagga ttcagataga actcggcctg gatgatcaca 240
tgttcttctt tgatagccca tgattcctga gcgctcatca gcacagctat gatgaaaaat 300
cctagcacag ggactccact tatggccatt ttcttcttgg gcgctctgtt gggagtcagt 360
agagctcgg 369

<210> 76
<211> 384
<212> DNA
<213> Homo sapien

<400> 76
acgactcggt gctcgccctg tccgcggcct tgcaggccac tcgagcccta atggtggtct 60
ccctggtgct gggcttcctg gccatgtttg tggccacgat gggcatgaag tgcacgcgct 120
gtgggggaga cgacaaagtg aagaaggccc gtatagccat gggtggaggc ataattttca 180
tcgtggcagg tcttgccgcc ttggtagctt gctcctggta tggccatcag attgtcacag 240
acttttataa ccctttgatc cctaccaaca ttaagtatga gtttggccct gccatcttta 300
ttggctgggc agggtctgcc ctagtcatcc tgggaggtgc actgctctcc tgttcctgtc 360
ctgggaatga gagcaaggct gggt 384

<210> 77
<211> 291
<212> DNA
<213> Homo sapien

<400> 77
acgtggcagc catggctccc ttcacaagct gtaggtcctg gtgggacagc tggctttggg 60
gaagcttgtc tttctgggtg acccatggat gctgcagaac ctgcttagct gtgaggcgct 120
ggtggggatc cacgtgtagc atcttggaca ccaggtcctt ggctgtctct gaaactgtgt 180
tccaatttcc cccactgagg gtaaacttcc cactgccgat ccgggttagg atttcctctg 240
gtgtgtcact gggaccgttg gcaaatggag tatatcctgc cagcatggtg t 291

<210> 78
<211> 242
<212> DNA
<213> Homo sapien

<400> 78
acccatattg ctaatgctag gatcaagata ccacatagcc agaacaagaa gttgaaggta 60
aacatagaat attttataca ggcactcaca cctgccattt cggaaaagga ttaggaatcc 120
agatgccgtg aatttaacta ttcgttacag gcttgtcctg caatatgctc tggagcaact 180
tgcctgcaga gatttctgta tccacggaca tttaaatatc gcaaaggcta tctccaggca 240
ag 242

<210> 79
<211> 449
<212> DNA
<213> Homo sapien

<220>

<221> misc_feature
<222> (1)...(449)
<223> n = A,T,C or G

<400> 79
ngtacagaca aaactacaga cttagtctgg tggactggac taattacttg aagganttag 60
atagagnatt tgcactgctn aanagtcact atgagcaaaa taaaacaaat aagactcaaa 120
ctgctcaaag tgacgggttc ttggttgtct ctgctgagca cgctgtgtca atggagatgg 180
cctctgctga ctcagatgaa gacccaaggc ataaggttgg gaaaacacct catttgacct 240
tgccagctga ccttcaaacc ctgcatttga accgaccaac attaagtcca gagagtaaac 300
ttgaatggaa taacgacatt ccagaagtta atcatttgaa ttctgaacac tggagaaaaa 360
ccgaaaaatg gacggggcat gaagagacta atcatctgga aaccgatttc agnggcgatg 420
gcatgacaga gctagagctc ggnccagcc 449

<210> 80
<211> 490
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(490)
<223> n = A,T,C or G

<400> 80
acatttcctt gnagactctg ntaatttcct gcagctcctg gttggttctg gagcagatga 60
tctcaatgag agagtcctcg tcggttccca gccccttcat ggaagctttt agctcanaag 120
cgtcatactg agcaggtgtc ttcaataggc ccaaaatcac cgtctccagg tggccagata 180
aggctgactt cagtgctgat gcaagttcct ttttggtcct tctctggtag gcgaaggcaa 240
tatcctgtct ctgtgcattg ctgcggntgg tcaaaatgtt gacaatggtg acctcatcca 300
cacctttggt cttgatggct gtttcaatgt tcaaagcatc ccgctcagca tcaaagntag 360
tataggcttt gacagaccca tatgcacttg gggtgtagag gtgatcaccc tccaagctga 420
gcttgcacag gatttcgtga acagtagaca ttttgaagga agctgggccg tgcgccgaga 480
gctgagagcg 490

<210> 81
<211> 339
<212> DNA
<213> Homo sapien

<400> 81
acagtagtaa ctgatgtccc cttcttcctg gatgaatgag cagataaata ttgatgtcag 60
catccttgaa ccatatcaaa gtgagcagtg tttggctact gcttctattt gaaatggtgc 120
tgtgttttgg ttgtggtctg aagctttgaa gcgctactta gcatctcctt tcttccatgg 180
agctctcacg attcaaacat gacagatttg gtaaaatgct ggttaggttg agtcttcctt 240
gcccccactc agtcatcttt gtatgaatcc catgatttgg gggttttttt cttttttttt 300
ataccagttt ttagctggtg tttatgaaga acagtgagt 339

<210> 82
<211> 239
<212> DNA
<213> Homo sapien

<400> 82
caagaacagc taaaatgaaa gccatcattc atcttactct tcttgctctc ctttctgtaa 60
acacagccac caaccaaggc aactcagctg atgctgtagc aaccacagaa actgcgacta 120
gtggtcctac agtagctgca gctgatacca ctgaaactaa tttccctgaa actgctagca 180
ccacagcaaa tacaccttct ttcccaacag ctacttcacc tgctccccc ataattagt 239

<210> 83
<211> 528
<212> DNA
<213> Homo sapien

<400> 83
acattcgtta ttttaaatga acaagtttac aaagtttatt ttcatctata cgtaaggatg 60
attttttttaa aactttttac atattagtgg ttatgatcca atgtgtcatg agtgaattta 120
actgtaaggt ggtttaaatc aaatatgcaa tgtttacttg aattgtattt ctattagcag 180
attttgacta tgtttacagg acggtttaaa ttaaggatta tcaggcatgt gagatctttc 240
agttatcttt aaagtagatg tatattaagg gcttagattt aggatctaca tattctgggc 300
attgaatagg cagtaactta caaataagtt ttgcttacct tttgttctag ggactagcac 360
tgctatcaat ggaaagtatt tttaactaat ctgttattaa gaaagtcata tttttgcatt 420
tcagccaaaa taaagaccgc ctgtaataat ctgttagaaa cagataatac atgtctgaaa 480
tccatatgtt tcatatgatc taaactgtat tttccaattt aaattaaa 528

<210> 84
<211> 249
<212> DNA
<213> Homo sapien

<400> 84
acactgaagc agaaccggaa acacccagga actgttcaga aatctcagaa gaaatctgct 60
tctcttcgat ggaaagatat aattaacgat caaagagctc taagaaaatt gcaaagaagc 120
cttaatgttc aagctttaga aagatcagag caatttttct ctttcagtcc aaactaagac 180
tctctgtatt taaatctctc tggggcaaga gggctagatt tcctcatttt gttatgagac 240
tagattggt 249

<210> 85
<211> 496
<212> DNA
<213> Homo sapien

<400> 85
actggccctc ggtgctggca aaggtgtagt tccactggcc gagggaatca agacatagtg 60
gtccttctgc taagccaagg gctgccacaa tgacacagta gccagatcct gcaattccaa 120
tgagagcagc caatacagaa gaaaacatcg cacatcgttt gccacagttt tcatggccac 180
agcagccaca gcagtcatcc tgttccagcc caatgaagac aaatgctggc aggagcatca 240
gcaggccacc tcctacgatg ccagaaaaga accacacgaa gcggctgagg tggttttcgg 300
aggcatactt tgtttcccca ttgggaaagt aaagcaaaat attaaccgcg atgcacagga 360
gggcgagccc caccagagaa tgtccgatgc atcgtgcaca cttcccatag cacatggtgg 420
tctgctaggt ttctccccc ttctctttgt cttcagctca gtgatacccc aaattagatg 480
aaagtgtgcc cttctg 496

<210> 86
<211> 199
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(199)
<223> n = A,T,C or G

<400> 86
acagaaagag taagataaaa acatttaata tnattaaatc taatttgcaa aaattggtat 60
ctgacatttg ttgtgtgctc ttgcaaagag cgcataggac atttctgcag caatcaaaaa 120

```
ggtaaaatct ttttaaactc agatttcaag tttcctctaa tattccttct aatcctantc      180
cctggaaata ctttcaagc                                                    199

<210> 87
<211> 436
<212> DNA
<213> Homo sapien

<400> 87
aacgttttga tttcatgaag gtgttctcaa atttaaagca cattttcagt aagaacaaaa       60
atatttaatg tttttatctt agacttaact tgatacattt gcatattact atggaagtta      120
ttcaccttgt ccctgttttt ctttaagata ttttaaaatc atagttatac tacagtcctt      180
ttttaaatgt atcctgatac attgtaaaat attttaattt cattgtggaa aataatgttg      240
gataaggaga tatttttcac tgttaacttt tagcccatgc attttcataa tttatttttt      300
tcacttgctg ctttatatga catatgtgac atttgattat ttaacacttg atgtgatctg      360
cataaaccca agttgcacaa ccctcctgct gaagataaaa ttgaggttaa agataaagat      420
ttattttcat atttgt                                                       436

<210> 88
<211> 596
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(596)
<223> n = A,T,C or G

<400> 88
acaaaagctg gtaatggacc aaagacttcc aaaatatatg tgtaatgacc tccagatttc       60
tttatagttg ttcccaattc agcataagac aaagctccaa atagtgacag gaccccacac      120
accgtccaga tggtcagaga catgcccacg ctgcccgtgt tctggagcac gcccttagga      180
gagatgaaga ttcctgctcc aatgatggtg ccaatgataa tggagactcc cctcagtaaa      240
gtgactttcc tcttcagctg cactttctcc tgcccaggtg gctccttgtt gcccagggaa      300
ggcagcctcc cgttaacatt tccctgcagg taacctcctt tggagatggt ggacacaaca      360
ggctttctga ccatagtagg gacacacggg ggaaaaataa aacagaggga aagaaaacaa      420
aactttcaac tttggtgtct cttggtgtta ctgatcgatg tcttcctctg ctttcagact      480
gtctctctca gcgctatagt gttcacaggt gaaaactcaa aggtgtgctt tttncttcac      540
agcgatctaa ttactactca gaaacacctg tgtatgcatc gtgctctcaa ttcttc          596

<210> 89
<211> 435
<212> DNA
<213> Homo sapien

<400> 89
acacaagtca gtccaacagt tagtgttaat tactaataat atatgaaaac cctgccaaca       60
caattgctgc tacatcacca atataattat taaccactgt cggaaaaaca cacataaatt      120
caggtaagac taaaagctgt ctcacaaaaa gaaaaaagaa atccaatgga tccactaatg      180
ctatcaaaag ggacatgcag gaatgtaaca tgacattttt agaaatgtgt gtttctaaaa      240
agaaaaaaaa atacactaaa atgccagtgg actataattc attcaaaaca tctttagtgt      300
tccttcccaa agatcttgat ctgctcagta attgcttcac aagatctatc acagccatct      360
tttggagcgt atggttaggc tggtcctcct gtggtggtag ggcagtcttt tttgaagctt      420
taagtatctg gtggt                                                        435

<210> 90
<211> 344
<212> DNA
```

<213> Homo sapien

<400> 90
actcagcgcc agcatcgccc cacttgattt tggagggatc tcgctcctgg aagatggtga 60
tgggatttcc attgatgaca agcttcccgt tctcagcctt gacggtgcca tggaatttgc 120
catgggtgga atcatattgg aacatgtaaa ccatgtagtt gaggtcaatg aaggggtcat 180
tgatggcaac aatatccact ttaccagagt taaaagcagc cctggtgacc aggcgcccaa 240
tacgaccaaa tccgttgact ccgaccttca ccttccccat ggtgtctgag cgatgtggct 300
cggctggcga cgcaaaagaa gatgcggctg actgtcgaac agga 344

<210> 91
<211> 371
<212> DNA
<213> Homo sapien

<400> 91
agcaatgcaa aggacatctc caatcatgac atttaagaca attctttatt tctctgacag 60
tgacttcttg aagtgcacat ataataaata aatagaaaat atatctttgt tcatggtgat 120
gcctacaaga aatgtttaca tacaaacact ctatacatct aactcccgaa aaaggaccag 180
ctatttcggc aacagaaaaa agacaagcat ttcagaggag cgttgctttc cttaaagacc 240
taactcactt aagtcttaca aacagaaata acaaggagga caattttcta agcaataaga 300
aaatttgtgc taccaagaaa atgcctagat attggctctt ggtgaatggt ttaggaaaga 360
aacttttatg t 371

<210> 92
<211> 209
<212> DNA
<213> Homo sapien

<400> 92
acaacaaaag atcaaaccca tgtcccgatg ttaacttttt aacttaaaag aatgccagaa 60
aacccagatc aacactttcc agctacgagc cgtccacaaa ggccacccaa aggccagtca 120
gactcgtgca gatcttattt tttaatagta gtaaccacaa tacacagctc tttaaagctg 180
ttcatattct tcccccatta aacaccagt 209

<210> 93
<211> 176
<212> DNA
<213> Homo sapien

<400> 93
actccctgtt ttgagaaact ttcttgaaga acaccatagc atgctggttg tagttggtgc 60
tcaccactcg gacgaggtaa ctcgttaatc cagggtaact cttaatgttg cccagcgtga 120
actcgccggg ctggcaacct ggaacaaaag tcctgatcca gtagtcacac ttcttt 176

<210> 94
<211> 494
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(494)
<223> n = A,T,C or G

<400> 94
aaatggaaat ttaantgaca tcctanaggt agagaaaccg nggagatcnc ttttctcaga 60
ctcaccaact tttaatggga tttcatgggg tttggttgtg ctgatagggt aaggggaggc 120

```
tgctttctgc ccttctcccc actcccatct gatttactta attcagtctc agctgctgaa      180
atttggaaag gaccaaattg ctttacagtt tttttctttg cgtagtatct tgaaatcctg      240
gaaaattcta tggaatagtt ctgtatatag ggcacaagta aaggcattgt ccaaagttta      300
tttatttatt tattacccta agaatgcttt gccataacca catttaatgg gaaaaacggc      360
annatcacag atgtaaatta nctcaccana tttactgngc ctgaactcat tctcttcttg      420
ctatatgatt tagcaagttc tagaaggnct ccaagacaat aattacattg gcacaatgta      480
tacttcagng ctca                                                        494
```

<210> 95
<211> 260
<212> DNA
<213> Homo sapien

<400> 95

```
cgcggcgagg tacgggcttt ccatctagtt gccagcttag atctggggtt ggtaacccac       60
tgactttgca gtccattctg cagagttttc cttcttgaac agtcagatct ccaggagcct      120
gcaagaagtg aggtctgaag aatcgctcct gaattggttc attttcgtct ccactgtccc      180
ttgatctaga acgaggcctt ctgacatgag gatggcctga gggagaccgg ggactccgac      240
ctctttggtt gacagcctgt                                                  260
```

<210> 96
<211> 438
<212> DNA
<213> Homo sapien

<400> 96

```
accagttctt gtttatatac agtagtgttt tgggcacacc taaggtcgat ctgtgttgta       60
tttaaaaatc taatttcttt atttgtgtgg ccttctagac aaacgaaggg gacccagagg      120
aaaccccctg acagatctct ggatgatcct ccttgaatcc tgggcagttt ggtctctcct      180
tgctgtgctc ctgtggcact aaactccttt tgattggttc tttctttcct tcccagctag      240
actaagcccc tcatgggcag gtaatgaaga ttgaaaactt ttttctgttc tccagtgtga      300
gcacattcct cctacatggt agatgtgcaa tagatgtttt taaaattgga gaatgaaaat      360
aaaagaagaa aatcacaatt tcttatcaag ttgtagcttg gtatcataca caattgcatt      420
ctgaggaatt aaggtggt                                                    438
```

<210> 97
<211> 454
<212> DNA
<213> Homo sapien

<400> 97

```
gagtaattcc cctccagcac tagagaccgc tcagtgctct tactagatga actcagtaac       60
gccttgagct gggttgattg aggatgtgtg aaaagctcac agagctcgat gcctgctgct      120
atttcacggc aatgagcctt tttctttcta cactgaagat tttcttctta tttaatgtgg      180
tttattttgg gctcagaaat aattgctctg ttgaaaataa tcctttgtca gaaaagaagg      240
tagctaccac atcattttga aaggaccatg agcaactata agcaaagcca taagaagtgg      300
tttgatcgat atattagggg tagctcttga ttttgttaac attaagataa ggtgactttt      360
tccccctgct tttaggatta aaatcaaaga tacttctata tttttatcac tatagatcat      420
agttattata caatgtagtg agtcctgcat gggt                                  454
```

<210> 98
<211> 226
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(226)

<223> n = A,T,C or G

<400> 98
actaaatggt ggtctaggag cagctgggcg natagcaccg ggcatatttt ggaatggatg 60
aggtctggca ccctgagcag tccagcgagg acttggtctt agttgagcaa tttggctagg 120
aggatagtat gcagcacggt tctgagtctg tgggatagct gccatgaagt aacctgaagg 180
aggtgctggc tggtaggggt tgattacagg gttgggaaca gctcgt 226

<210> 99
<211> 333
<212> DNA
<213> Homo sapien

<400> 99
actcatctag acgtttaggt atttttcgtg gttgaggaag ctcctctact aaattcttaa 60
gaatatcttc tggaatatac tcatctggaa aaagatgcaa cctttccatc attgttcttc 120
tgtgaaggtt ttttggcagc atgccataaa tagctagttt tacaattgcc actggatccc 180
tcaggtgaag ctgagcagct gttacttgtc taaatccacc tgggtagcca gtatgcgaag 240
agtatacttt ttgttcccat ttgtttccag aaaatgcaat gtgtcttgtg ttcattataa 300
caacatgatc cccacagtca ctcagtgcat ggt 333

<210> 100
<211> 417
<212> DNA
<213> Homo sapien

<400> 100
accgccacat cgctgacttg gctggcaact ctgaagtcat cctgccagtc ccggcgttca 60
atgtcatcaa tggcggttct catgctggca acaagctggc catgcaggag ttcatgatcc 120
tcccagtcgg tgcagcaaac ttcagggaag ccatgcgcat tggagcagag gtttaccaca 180
acctgaagaa tgtcatcaag gagaaatatg ggaaagatgc caccaatgtg gggggatgaag 240
gcgggtttgc tcccaacatc ctggagaata aagaaggcct ggagctgctg aagactgcta 300
ttgggaaagc tggctacact gataaggtgg tcatcggcat ggacgtagcg gcctccgagt 360
tcttcaggtc tggaagtat gacctggact tcaagtctcc cgatgacccc agcaggt 417

<210> 101
<211> 438
<212> DNA
<213> Homo sapien

<400> 101
acatatgttt tttaagtaag ttacttttac cattagaata aacctagaca ctacagggac 60
aactctgggg aacagggcgg tctgccttaa caacccttct ctaggttgag gaaggcaggt 120
atagttcact gaaggatgtg atgaggctgt agtaagtctt ctcatcatct gttaatcctg 180
cgttgcctgg tctcaccacc acagctacgt gcacatctgc ttcctcagca gcactggcct 240
ctcgagtaac atctgtcaga aacaaaatgt tgttggttga gcacccaatg ctgtctgcaa 300
tctttcggta actttcactc tctactttgt gtccaatctt ggtatcaaag tgaccatcaa 360
caagctcaag aatatctccc tccgtagaat gcccgaataa cagtttctgt gcctccacac 420
tccctgagga atagatgt 438

<210> 102
<211> 466
<212> DNA
<213> Homo sapien

<400> 102
acttaaaaag tggtttttct atcttcaaag tgctaaagaa acaagtattc aaaaagaaac 60
ttcaggtcgg tctacgaagt tctgactgac ttgaagtagt gaaataccaa gaatgcagtg 120

gacaaattta aaaggccttc attagaataa agtatatctt aactacattt tgcaaagaaa 180
tgaagcaatg gttgcacaac cagtcagggc caagttagta acatacaact cagccatcag 240
cccacctctc cctcaaacta aactaatcta aatgtatttt tcagaaaatt tcctccatac 300
tccatgtatg tgttacatac atccaatcat atccatattt tggatcattt ttttctatat 360
tcatcagatt attggttaaa atgcacagca agtagaaatg atccatttca aaattcttaa 420
tatctagcgt tctctgtaaa acaaaagctg acaacagttt tattgt 466

<210> 103
<211> 500
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(500)
<223> n = A,T,C or G

<400> 103
nggtgcagcg gagacagagg cggaagctgc agccctagag gtcctggctg aggtggcagg 60
catcttggaa cctgtaggcc tgcaggagga ggcagaactg tcagccaaga tcctggttga 120
gtttgtggtg gactctcaga agaaagacaa gctgctctgc agccagcttc aggtagcgga 180
tttcctgcag aacatcctgg ctcaggagga cactgctaag ggtctcgacc ccttggcttc 240
tgaagacatg agccgacaga aggcaattgc agctaaggaa caatggaaag ggctgaaggc 300
cccctacagg gagcacgtag aggccatcaa aattggcctc accaaggccc tgactcagat 360
ggaggaagcc cagaggaaac ggacacaact ccgggaagcc tttgagcagc tccaggccaa 420
gaaacaaatg gccatggaga aacgcanagc agtccanaac cagtggcagc tacaacagga 480
gaagcatctg cagcatctgg 500

<210> 104
<211> 422
<212> DNA
<213> Homo sapien

<400> 104
tggttctagg agatatcaat accaaaccaa agaaagaaaa tattatagct tttgaggaaa 60
tcatgaagtc tgtatggctc aatgatttcc tgaagatgat aaagagcaag atattgcaga 120
taaaatgaaa gaagatgaac catggcgaat aacagataat gagcttgaac tttataagac 180
caagacatac cggcagatca ggttaaatga gttattaaag gaacattcaa gcacagctaa 240
tattattgtc atgagtctcc cagttgcacg aaaaggtgct gtgtctagtg ctctctacat 300
ggcatggtta gaagctctat ctaaggacct accaccaatc ctcctagttc gtgggaatca 360
tcagagtgtc cttaccttct attcataaat gttctataca gtggacagcc ctccagaatg 420
gt 422

<210> 105
<211> 326
<212> DNA
<213> Homo sapien

<400> 105
acgaagtagg tccaaagttg ttgaccgtat ttacagtctc tacaaactta cagctcataa 60
acataaaatg aatactgaaa gaatacttta caagcaaaag aagaattctt ctataagcat 120
tccttttatc ccagaaacac ctgtaaggac cagaatagtt tcaagactta agccagattg 180
ggttttgaga agagataaca tggaagaaat cacaaatccc ctgcaagcta ttcaaatggt 240
gatggatacg cttggcattc cttattagta aatgtaaaca ttttcagtat gtatagtgta 300
aagaaatatt aaagccaatc atgagt 326

<210> 106
<211> 543

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<212> DNA
<213> Homo sapien

<400> 106
acttgtaatt agcacttggt gaaagctgga aggaagataa ataacactaa actatgctat      60
ttgatttttc ttcttgaaag agtaaggttt acctgttaca ttttcaagtt aattcatgta     120
aaaaatgata gtgattttga tgtaatttat ctcttgtttg aatctgtcat tcaaaggcca     180
ataatttaag ttgctatcag ctgatattag tagctttgca accctgatag agtaaataaa     240
ttttatgggt gggtgccaaa tactgctgtg aatctatttg tatagtatcc atgaatgaat     300
ttatggaaat agatatttgt gcagctcaat ttatgcagag attaaatgac atcataatac     360
tggatgaaaa cttgcataga attctgatta aatagtgggt ctgtttcaca tgtgcagttt     420
gaagtattta aataaccact cctttcacag tttattttct tctcaagcgt tttcaagatc     480
tagcatgtgg attttaaaag atttgccctc attaacaaga ataacattta aaggagattg     540
ttt                                                                   543

<210> 107
<211> 244
<212> DNA
<213> Homo sapien

<400> 107
acaaaaatgg ttataaaatg gttgaagcaa ctagaagcgt gacaggtata atacatataa      60
atacaaccaa aattcaattc aatgcaaagt tgaatgacat catattgcac caaaatttat     120
tccatacaaa agcacatgca tcaagagttt tcataagatg aaaacaaaca cacttacttc     180
atagcatctt accacttact tacacaaata gcccataaac accatctggc attgtgattg     240
cagt                                                                  244

<210> 108
<211> 511
<212> DNA
<213> Homo sapien

<400> 108
acttcatgtg atttgtcaac catagtttat cagagattat ggacttaatt gattggtata      60
ttagtgacat caacttgaca caagattaga caaaaaattc cttacaaaaa tactgtgtaa     120
ctatttctca aacttgtggg atttttcaaa agctcagtat atgaatcatc atactgtttg     180
aaattgctaa tgacagagta agtaacacta atattggtca ttgatcttcg ttcatgaatt     240
agtctacaga aaaaaaatgt tctgtaaaat tagtctgttg aaaatgtttt ccaaacaatg     300
ttactttgaa aattgagttt atgtttgacc taaatgggct aaaattacat tagataaact     360
aaaattctgt ccgtgtaact ataaattttg tgaatgcatt ttcctggtgt ttgaaaaaga     420
aggggggag aattccaggt gccttaatat aaagtttgaa gcttcatcca ccaaagttaa      480
atagagctat ttaaaaatgc actttatttg t                                    511

<210> 109
<211> 652
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(652)
<223> n = A,T,C or G

<400> 109
acaccccaaa ctctcccttg ggagcctcaa tggcagtata tgtggctcct ggaggaactt      60
ggtagccctc agtatacaac ttaaagtgat gaatcagtga ctccatggaa gtcttcatct     120
ctgctcgctt aggtggagac actttggcat catcaacctt gatctcccca ggaggcatct     180
tgtttagaca ctgtgcgata attctcaggg actggcgcat ctcctccacc cggcacaggt     240
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acctatcata gcagtcccct cgagaaccaa caggaacatc aaactcaacc tggtcgtaaa 300
catcataggg ctgggtcttc cgcaggtccc actggatgcc tgagccccga agcatcactc 360
cactaaaacc atagttaagt gcttcttctg ctgttacaac cccaatgtca attgtccgat 420
ttcgccagat cctattgttg gtcagcaact cctccaactc atcaagccga agagagaagt 480
tcttagaaaa ctgataaatg tcatccataa gcccaagggg taggtcctgg tgcactcctc 540
ctggccggat ataagcagca tgcattcggg ctncagacac ttcgctcgta gaactcaaac 600
atcttctncc tttcttcaaa cagccagaag aaaggggtca tgggcccaag gt 652

<210> 110
<211> 96
<212> DNA
<213> Homo sapien

<400> 110
acacattgag tattccacag atatacatgg tttaatatgt ggtatccatg gggtatgatt 60
ctaccacagc cttgtaagtg ctccaaacct taaagt 96

<210> 111
<211> 371
<212> DNA
<213> Homo sapien

<400> 111
acatagcagc ttcataacag tttacttttt taatataaag atttttcaat ttacacttgt 60
aggagtagaa aaaactaata tgctaagtct gtaagctacg cagcaaaaat aatgatctta 120
atgaagccag aattctgtga aaatgtgcac cacactgcat atatagtagc tgagtaaatg 180
taaaccatgt gcttattaac tcttctatat aaaatattga acccccaagt ctcacacatt 240
gcctcctatg tccacatcac ttttctgaag acagcctcat gctttaagcc aatatatatt 300
tgctatttga aaaagttctc atcctcatta ctaaaaatgt ttctgtaaag gccttagaca 360
ttttttcag t 371

<210> 112
<211> 406
<212> DNA
<213> Homo sapien

<400> 112
caggtacagt aatacacggc tgtgtcctcg gttttcaggc tgctcatttg cagaaacaac 60
gtgtcttctg aatcatctct tgagatggtg aatctgcctt gcacgggtgc agcgtagtct 120
gttgtcccac catcagttgt gcttttaata cggccaaccc actccagccc cttccctgga 180
gcctggcgga cccagctcat ccaggcgtca ctgaaagtga atccagaggc tgcacaggag 240
agtgttaggg accccccagg ctttactaag cctcccccag actccaccag ctgcacctca 300
cactggacac catttaaaat agcagcaagg aaaatccagc tcagcccaaa ctccatggtg 360
agtcctctgt gttcagtcct gatcactgaa tgaaaacact tgggaa 406

<210> 113
<211> 492
<212> DNA
<213> Homo sapien

<400> 113
accatcccca gaagtgtctg gtgccaggca ctgatccagc agctcttcca caatggatga 60
caataaccga agctccccat tttcatcacg ctggctgatc tttgattgaa tgaaatctac 120
aacttcctgg ctgctcatca cattccagat gccatcacag gcaatgacca tgaattcatg 180
gtcgtcagtg agagtcagca ccttgatgtc aggaagggct gaaatcatct gttcctcagg 240
tggcaggttc ttgtttctct tgtagaagtg gtccccaatg gctctggaga ggttgaggcc 300
cccgttgact cgcccatcca tggtgacctt gccaccagca ttcttgatgc gtgctagttc 360
tacttcatcc tctggtttgt gatcatagga catgtctaaa gctttgccag cctcagatac 420

cacacagcga gagtctcctg cgttggctac aatcaactgc ttctctcgta tcagggccac 480
caccgctgtt gt 492

<210> 114
<211> 234
<212> DNA
<213> Homo sapien

<400> 114
acctcagtgc aaaagttagt tgaactggtt cattcatctc tatggtaaca gcttcctcct 60
ctttatcgac attacttgtc tgtgacaatt taatgtttcc atttccaagt tctccacttg 120
cagaaaattt cactccgtct tttgcacagg aaattacaac agcatctcca atatggctga 180
gatctcggca tatacgtgca aattcaccag aaggcatctt tactacacag ctgt 234

<210> 115
<211> 368
<212> DNA
<213> Homo sapien

<400> 115
cctggggtgg gatcagagga tctggcgtgg catcccgtag ccagtcatgc ctgcctgaga 60
cgcccccgcgg ttggtgccca tctgtaaccc gatcacgttc ttgccctctt gcagctggtt 120
atccgagaag ttccgaggat tctccttgga tttcttaggg aaccagttgg gatccccaga 180
gaagagccca tcatctcggg ctactgccag cccacccaga ttcatcagcg tccgctgcac 240
acaggccatg ttctttcctt cccagaggtc cacagtttgg aagatgtcag tggtgttaat 300
gccatagcgc tcagctgctt gcaggaactg agagatctgc tccatctgct tgaaggccat 360
ggtggagg 368

<210> 116
<211> 487
<212> DNA
<213> Homo sapien

<400> 116
ggatttttta ttgtgttttc cacatagata aaaaaataag gctttttgat gaaaagaatc 60
cattacaaag tcaaaaatcc attacaatta taattgaatc agtaacaaaa tttagcttta 120
aatgagtcaa gtattctgca tttgaaattt aatatcacaa acattcaaga ttagtgaatt 180
ttggtaagaa aaaaatacta gaagaaagga aaaggacacc ttttcaacag atagtaattt 240
ataaaaattt ttttaaaagt gctttgggaa aacacacagt atcattactt aagaaaagtc 300
atttaaggaa gacttaagtg cttcaagtgg agtgtattac agactaaaaa atgttttaaa 360
atttgccaag aaatttaagt gttaaaaata ctcttctcct tattcagttt catgtttaag 420
gaaacatttg acagacaagt aaaccaaacg caaaaaaaag ttcacctgca ttttaaacta 480
ataaatt 487

<210> 117
<211> 430
<212> DNA
<213> Homo sapien

<400> 117
gttttacttg ttgatttttg gatgcatgct gggggaggaa agcatattgt ttgtagtcac 60
cctagagtgc taaggtatat tattccccag taattctctc aaggtgggca tatgcaaaac 120
ataatctcta aattcttcaa tactaagaaa tacctttgtt ttacccctaa aatcaaatgc 180
cattttggct ggatatagga ttctaggatt aaagcctttt tccagcagaa ctttgaagac 240
attgctccat ttacttctag catccagtgt gtccagtgat aagtctgctg tcaacctgat 300
tcttgttcct tggtaggtaa tttctcttct ctctctagaa gcccttatta ttttctcttt 360
atcactagaa ttccaaaatt tcaccaagat gtgtctagga gtcagtctct tttcatcaat 420
tttactaggt 430

<210> 118
<211> 305
<212> DNA
<213> Homo sapien

<400> 118
cctgctagaa tcactgccgc tgtgctttcg tggaaatgac agttccttgt ttttttgtt 60
tctgtttttg ttttacatta gtcattggac cacagccatt caggaactac cccctgcccc 120
acaaagaaat gaacagttgt agggagaccc agcagcacct ttcctccaca caccttcatt 180
ttgaagttcg ggtttttgtg ttaagttaat ctgtacattc tgtttgccat tgttacttgt 240
actatacatc tgtatatagt gtacggcaaa agagtattaa tccactatct ctagtgcttg 300
acttt 305

<210> 119
<211> 367
<212> DNA
<213> Homo sapien

<400> 119
cggtacaaga catcaaagtg aagtaaagcc caagtgttct ttagcttttt ataatactgt 60
ctaaatagtg accatctcat gggcattgtt ttcttctctg ctttgtctgt gttttgagtc 120
tgctttcttt tgtctttaaa acctgatttt taagttcttc tgaactgtag aaatagctat 180
ctgatcactt cagcgtaaag cagtgtgttt attaaccatc cattaagcta aaactagagc 240
agtttgattt aaaagtgtca ctcttcctcc ttttctactt tcagtagata tgagatagag 300
cataattatc tgttttatct tagttttata cataatttac catcagatag aactttatgg 360
ttctagt 367

<210> 120
<211> 401
<212> DNA
<213> Homo sapien

<400> 120
acaggtaaat aaaagatcac cttgaattaa actggatctc cttaagggca tagtatagtt 60
tcagtttcat tacctattac ataattagtt tcttacatac aaatattgac atatttggct 120
tgtgcttcga agcctttgtg tctatgaagt ccacatcaat gcagctcata actggaagtc 180
actgggggagt tctttgctgc tgctgggttt aacctgatca tgcattagag tctcctcagc 240
acctgttgtg gctctgcaca cctctggggc atcgtcagtg tcaggatcca agccttcagg 300
gcagggaagt ttcagcaact cttcgcggag ctgagcagtg tgacgcttga gagctgctgc 360
atggtgagac atagtcctgc ctacccgctt atcactgctg t 401

<210> 121
<211> 176
<212> DNA
<213> Homo sapien

<400> 121
acagcccaga tgtgatattt ctacaggaag ttattccccc atattatagc tacctaaaga 60
agagatcaag taattatgag attattacag gtcatgaaga aggatatttc acagctataa 120
tgttgaagaa atcaagagtg aaattaaaaa gccaagagat tattcctttt ccaagt 176

<210> 122
<211> 443
<212> DNA
<213> Homo sapien

<220>

<221> misc_feature
<222> (1)...(443)
<223> n = A,T,C or G

<400> 122
actgctgcca gttccccacg tggcccagcc ccacccacag gctctcctgg gcccaggaat 60
gtcctgcagg agggaggagt cggtttccaa tgccagccgc cctaacaacc caggaactca 120
gctcaactgg ttacagacct cgagttttca gcccatgtta cttgaaggag aagcagttct 180
tgggctttac cacctgccac ctgggccaga gttctcttat ccttatccta agagtcttta 240
agactcaaag aagaaaaggt cttgtctgat gtataatctt aaaataaacc cacacttagc 300
cacctcaaat cctttctgaa attatgtaag atgaaaactt aaatgcctta tagataccaa 360
gtatctcctc acaatattga attccatgaa accacttatc tttgcatgca atgaagcatc 420
cacaaaacca tttcaagctg aan 443

<210> 123
<211> 520
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(520)
<223> n = A,T,C or G

<400> 123
actgtatatt ngaagattgc taagataatg gattttaagt gatctcacca caaaaaaaga 60
agtatataag gtattagata tgttaattag cttgatttag ttattctaca aggtatccat 120
atatcaaaac atcatgttat ataccatgaa tatagacagt ttctgtcagt taaaagtaaa 180
taaaaatttt aaaaaattat caattcgtta attttaccaa gttggggcaa aagcctttta 240
acagtccang aaatatttaa agctagtcaa cagcttctac agagatgaag aacattntgt 300
cctaaggggt ttctgtaggg atcacccca tctctagact tctacctggt aaacacgcct 360
tccactgggt gatgaganta aggtgatgga ctgtcgatca actaggncca aggcctgggt 420
agctgatgag ccaaagagaa acttcagcct gtgaaataaa aacacttcag attagaangc 480
ctgattctca aagtcacctc agtaacttgc ccaaggatcc 520

<210> 124
<211> 406
<212> DNA
<213> Homo sapien

<400> 124
actaaaaatc aattggatga actaaatcca aaacatgaca ctgtaggcag cagttttaag 60
tcttattttt actgtttata tatttgaatg ctgctacaac agatgatctt catccctgaa 120
gttttcagct aaacttggtt tcctagaata gactgttaac tttcaaaatt tttattggtg 180
aaatggaaat actgtttttc cttgtgaatg aattttcata tttgtaagtg ctaagtttat 240
aattcaggtt tgatcaaggt gtgaataact gaagaaaata acttgctggc tatataggaa 300
aatgctgtgg aaatgaactg tgtatatact tctgggagga acaaatttaa tcatttcttc 360
tgttaagcac taatcagtat aagtgcaact cctggttctg tacctg 406

<210> 125
<211> 413
<212> DNA
<213> Homo sapien

<400> 125
gttttctttg aatgatttct ttttttcact gtaagacact cctttaaata atgcctatct 60
ttaacttttt aagactattt ggaaaaatgc agtgtctcag ctgtccccag ggaaattaag 120
tggaattcaa ctaagatctg ttaataagat gtcagaataa ctaataattt tattaggaaa 180

aaatcatgtt ttaaatttca aaatgacact tatttgtcaa gtaatatgat cttggaaaat 240
tttaaagaaa aataatccta cttataaact actttttat aattgttttc agaaaaaaag 300
tttacagtct taaggaaaat attcaggtct atcatatggt ttgacagatt ttttaaaagt 360
tatttttggt aaggtcttct tttagaaaaa aattaatctc aagggttttt tgt 413

<210> 126
<211> 655
<212> DNA
<213> Homo sapien

<400> 126
gtattctata gtgtcaccta aatagcttgg cgtaatcatg gtcatagctg tttcctgtgt 60
gaaattgtta tccgctcaca attccacaca acatacgagc cggaagcata aagtgtaaag 120
cctggggtgc ctaatgagtg agctaactca cattaattgc gttgcgctca ctgcccgctt 180
tccagtcggg aaacctgtcg tgccagctgc attaatgaat cggccaacgc gcggggagag 240
gcggtttgcg tattgggcgc tcttccgctt cctcgctcac tgactcgctg cgctcggtcg 300
ttcggctgcg gcgagcggta tcagctcact caaaggcggt aatacggtta tccacagaat 360
caggggataa cgcaggaaag aacatgtgag caaaaggcca gcaaaaggcc aggaaccgta 420
aaaaggccgc gttgctggcg tttttccata ggctccgccc ccctgacgag catcacaaaa 480
atcgacgctc aagtcagagg tggcgaaacc cgacaggact ataaagatac caggcgtttc 540
cccctggaag ctccctcgtg cgctctcctg ttccgaccct gccgcttacc ggatacctgt 600
ccgcctttct cccttcggga agcgtggcgc tttctcatag cttcacgctt gtaag 655

<210> 127
<211> 442
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(442)
<223> n = A,T,C or G

<400> 127
accttatggt ccttgaaagg aagactcaat acttccagga gtcaaagtta atttgaatga 60
aaatggaaga gaacaagttg acaataattt gaagcaattc atgcttctag ggctgaatga 120
cgtttagatc agacacagag tgactgagcc aatcaacagg catgtagtgt gatctttccc 180
accacagtga acagagggat tctttgtcca aggcaggctt gcagctcggt ccagcttgag 240
catttgatca ggatttgatg cttcaaagat gacccactct ctgtaaactc attaccaaag 300
caaaatgcaa tgatctcttc catttgtgga acataccacc aacacaaacc acgcgtggct 360
ttgcctcctg ttcactccat tttcaaggct agagaaagtt caagtccaaa acaacagtta 420
aggntaaaac gctaaacctc aa 442

<210> 128
<211> 447
<212> DNA
<213> Homo sapien

<400> 128
gtaaaatctg atggtggtta aatgacgatg tttaggtttt gataaattta gattttatac 60
acatgataga gcatgtatct gtatttttaa aaataaagac agagaactta tgtttagaac 120
aagagaagcc atttggtaga aataaagaag gagattgggg aaggagatga gaatgagtca 180
gagagatagc atttaaaact tgaaatcagg cacaacaatt agtatgtcat gatataaaca 240
gtattgagat aaaattttac cacttctctt ccctttaata aattgtcaaa ggataaagtt 300
tcctgtttga aaatatattt tactggtatt gtgctttcct catatcacag attggtaaag 360
aatcatttta agtccaagac tcttatttta catattctgc aattaaaggt cctatgaggc 420
tacctgccga ctgctgacat gtagtgt 447

<210> 129
<211> 175
<212> DNA
<213> Homo sapien

<400> 129
ttcagacttt gtttgtagtc agccttggtt tggcttcaga ctttgtttgt cgtatttgag 60
gatataaata ttcatgaata gtttcccaag tctggagcga ccacataggg agaaaatgta 120
aatgtctcaa tttttgttca caaaagtata ttttatcaaa ttgctgtaag ctgtg 175

<210> 130
<211> 406
<212> DNA
<213> Homo sapien

<400> 130
acatttacat tcaagttgat aacactggtg gtttcatttc aatacaaatt atgctagaga 60
actgacattt cagacatggt catatatatg ctatttgaat tcctttatct tgatacagat 120
cttgattgtg aatctcttga tgatagatgt gcagctaatt tgtcccgaaa ctcatgaaga 180
taattgtatt gcttgatggt ctgtattgcc ccggatcctc ttaggtctcg caggctgtct 240
atggcttgct ctggtgatat tgtgtcagac aggtatagta ggagacaagc agctacaaga 300
caagatctcc caagtcctcc atagcagtgt attaaggttt ttcggtaatt tttaaggcag 360
gttgtaagct cttccattat ttcacagcag ctggctatgt caggag 406

<210> 131
<211> 403
<212> DNA
<213> Homo sapien

<400> 131
accgcattac attatgcctg tgaaatgaaa aaccagtctc ttatccctct gctcttggaa 60
gcccgtgcag accccacaat aaagaataag catggtgaga gctcactgga tattgcacgg 120
agattaaaat tttcccagat tgaattaatg ctaaggaaag cattgtaatc cttgtgacca 180
caccgatgga gatacagaaa aagttaacga ctggattcta tcttcatttt agacttttgg 240
tctgtgggcc atttaacctg gatgccacca ttttatgggg ataatgatgc ttaccatggt 300
taatgttttg gaagagcttt ttatttatag cattgtttac tcagtcaagt tcaccatggc 360
cgtaatcctt ctaagggaaa cactaaagtt gttgtagtct cca 403

<210> 132
<211> 479
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(479)
<223> n = A,T,C or G

<400> 132
cgaggtacag ggggaccccc ttctcaacgg caccagcttt gcagacggca agggacaccc 60
ccagaatggc gttcgcacca aacttagatt tattttctgt tccatccatc tcgatcatca 120
gtttgtcaat cttctcttgt tctgtgacgt tcagtttctt gctaaccagg gcaggcgcaa 180
tagttttatt gatgtgctca acagcctttg agacaccctt ccccatatag cgagtcttat 240
cattgtcccg gagctctagg gcctcataga taccagttga agcaccactg ggcacagcag 300
ctctgaagan accttttgag gtgaagagat caacctcaac agtgggattc ccgcgagagt 360
caaagatctc cctggcatgg atcttgagaa tagacatggt gaacttctag ccactgggtc 420
tcgtcgccta ggagaggaag cggagggtgc tgcanacacc gaggtgaacg taaagcccg 479

<210> 133
<211> 301
<212> DNA
<213> Homo sapien

<400> 133
gtcttacagt gtgactcaga ctccctatct ggggatcggt taggttgctt caatctaact 60
atcaaaggac acgccaagtg tgtggaattt gtcaagagct ttaacctgcc tatgctgatg 120
ctgggaggcg gtggttacac cattcgtaac gttgcccggt gctggacata tgagacagct 180
gtggccctgg atacggagat ccctaatgag cttccataca atgactactt tgaatacttt 240
ggaccagatt tcaagctcca catcagtcct tccaatatga ctaaccagaa cacgaatgag 300
t 301

<210> 134
<211> 494
<212> DNA
<213> Homo sapien

<400> 134
actaagtgta tacgtatttt tgccactttt tcctcagatg attaaagtaa gtcaacagct 60
tattttagga aactgtaaaa gtaataggga aagagatttc actatttgct tcatcagtgg 120
taggggggcg gtgactgcaa ctgtgttagc agaaattcac agagaatggg gatttaaggt 180
tagcagagaa acttggaaag ttctgtgtta ggatcttgct ggcagaatta actttttgca 240
aaagttttat acacagatat ttgtattaaa tttggagcca tagtcagaag actcagatca 300
taattggctt atttttctat ttccgtaact attgtaattt ccacttttgt aataattttg 360
atttaaaata taaatttatt tatttatttt tttaatagtc aaaaatcttt gctgttgtag 420
tctgcaacct ctaaaatgat tgtgttgctt ttaggattga tcagaagaaa cactccaaaa 480
attgagatga aatg 494

<210> 135
<211> 448
<212> DNA
<213> Homo sapien

<400> 135
actgaactcc catcacaaca tcatcttcct ctaataactg taacacaaca ccttcaataa 60
actttgcatt gggctctgcc atagctgctt tccggagact catgatgaat cttccgtgat 120
ggaaagctct tccactctgc acttgattgt tttctgacag agggtaagga atctgaacct 180
ctgatttgct ttcctgatca tgaatcatgt aaccatttac aacctgggca tcaagacctt 240
ccactgtatc tccaagacca aggtctttga gaacatgata accaccccggc tgcaggaatt 300
ctccaactat tctgtcaggc tcttttaagt ctctctcaat gactgtcacc tttcttccat 360
ctctggaaag cacagctgcc aaagcagagc caagcacgcc agctcccacg atgataactt 420
ctgggtcatt ctgagaagat gttgatgt 448

<210> 136
<211> 527
<212> DNA
<213> Homo sapien

<400> 136
accatggtgt cagcaatttc ttccataact tcgtggtaat ggtaattaaa agccatttca 60
atgtccaaac caacaaactc agttagatgt ctatgggtat tagagtcttc cgctctgaat 120
actggtccaa tagagaaaac cttctcaaaa tcagcacaaa tgcacatttg cttatatagc 180
tgtggggact gagccaggta tgcattattt ttaaaatatg acacagtaaa aacattggct 240
cctccttcac tggcagctga aataatttta ggagtttgga tttccacaaa acctttgtta 300
attaaagttt ctcggaagag atggcagatg ccagactgga gacggaagac tgcctgacta 360
gttgatgtcc taagatcaat gactctgttg tctaatcttg tatcctggtt aacagtagct 420
cttccttcct cttctccttc tgcctcaggc cgaacagcat catccagctg caggggcaga 480

cggggttcag ccaaactgat cacataaatc ttctgaacat gtaactc 527

<210> 137
<211> 275
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(275)
<223> n = A,T,C or G

<400> 137
acgacgagtc gggcccctcc atcgtccacc gcanntgctt ctaaacggac tcagcagatg 60
cgtagcattt gttgcatggg ttaattgaga atagaaattt gcccctggca aatgcacaca 120
cctcatgcta gcctcacgaa actggaataa gccttcgaaa agaaattgtc cttgaagctt 180
gtatctgata tcagcactgg attgtagaac ttgttgctga ttttgacctt gtattgaagt 240
taactgttcc ccttggtatt tgtttaatac cctgt 275

<210> 138
<211> 354
<212> DNA
<213> Homo sapien

<400> 138
caagctcaag gtgtttctgt caggaatgcc agagctgcgg ctgggcctca atgaccgcgt 60
gctcttcgag ctcactggcc gcagcaagaa caaatcagta gagctggggg atgtaaaatt 120
ccaccagtgc gtgcggctct ctcgctttga caacgaccgc accatctcct tcatcccgcc 180
tgatggtgac tttgagctca tgtcataccg cctcagcacc caggtcaagc cactgatctg 240
gattgagtct gtcattgaga agttctccca cagccgcgtg gagatcatgg tcaaggccaa 300
ggggcagttt aagaaacagt cagtggccaa cggtgtggag atatctgtgc ctgt 354

<210> 139
<211> 527
<212> DNA
<213> Homo sapien

<400> 139
acgaggaatg acctctaggg cctgggcaac agccctgtat ggccattgtt ccacaccagt 60
catggccttg gatttttctg tcaaggcatg ggccacagcc atctcggagg ccccaccccc 120
tggcaccagc tgagggtcca ggagaacatt gcgacacact tgcatggcat cctggaggtt 180
gcgttctact tccgagagaa tctctttgct agcccccccgg aggagaatgg tgcaggcctt 240
ggggtctttg cagtcagtga tgaaagtaaa gtattcatct ccaattttct tgatttccaa 300
caggcctgct cctgttccaa catcatcttc tctcagttcc tctggtcggc tgactatccg 360
ggccccacag gctctagcaa tgcgattatt gtctgtcttc cggactctgc ggatggctgt 420
gatattggcc cgcataaggt agtgctgagc taaatctgag atgccctttt cagtgatgac 480
cacatcgggc ttcagttgga taatgtcctc acagagctgc tggatgt 527

<210> 140
<211> 396
<212> DNA
<213> Homo sapien

<400> 140
acgccactgt ctcttagata taattatccc caccctctgc tcatttgttt cccagattca 60
atacattgtc aaagcctctt ggtccttttt taacatctca cacttgtgtc attctctcca 120
ttcccataaa cctcaacaac tgctcaaagt cctgcttgac cccttgttgc cagtctttga 180
aatctttctt gcatatgact gcctcattac cttcctaaaa tctagttcac tcgcctactc 240

aagaagacac aggggcctac tgtggtgtat tagataagtt cacatttctt ctctttacta 300
atctttttta cttcctttac caccactccc ttatataatt ccatcatcct aatagatctg 360
tttccctaca catccctgcc tctccacccc acatgt 396

<210> 141
<211> 490
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(490)
<223> n = A,T,C or G

<400> 141
acaaccagct gtgctataag aaagagggag ggcctgacca taactacacc aaggagaaga 60
tcaagatcgt agagggaatc tgcctcctgt ctggggatga tactgagtgg gatgacctca 120
agcaactgcg aagctcacgg gggggcctcc tccgggatca tgtatgcatg aagacagaca 180
cggtgtccat ccaggccagc tctggctccc tggatgacac agagacggag cagctgttac 240
gggaagagca gtctgagtgt agcagcgtcc atactgcagc cactccagaa agacgaggct 300
ctctgccaga cacgggctgg aaacatgaac gcaagctctc ctcanagagc caggtctaaa 360
tgcccacatt ctcttnctgc ctgctgttcc ttctccttta tggacgtcta gtccttgtgc 420
tcgcttacac cgcaggcccc gcttctgtgt gcttgtcctc ctcctcctcc caccccataa 480
ctgttcctaa 490

<210> 142
<211> 511
<212> DNA
<213> Homo sapien

<400> 142
acatccagtc tgtatttctt acacaaaatt acatctaaat atttgacatg aggtcatttg 60
ctatcataag ccatcactag gaacttctag tctgtctcac tcgattgagg ctacaatgtt 120
gttaggtgct atgaccacaa tgaatacaac agacagcctc tcagctgtgc tgcaaagtat 180
tcataaccaa aagaccatat ttcaaattaa atcatagtag cgaatgacat accatttaca 240
tattacaatc tgagcctctg aaacaggggg aacatataat ggtatccaga acatctttac 300
atcaaaataa cctatcatac tacaaagttt tcacttccaa aaagtgtaac agagtttaag 360
gcactggtaa ctttgtccac tgttagagat taaaacttcc aaagcaaatg aaagaaccaa 420
tgttcacctt taacgtgggg aaagttggca aaaagaaccc caggaggaca cccaaacctt 480
ctctgtgtcc tctgtggaac ctggcttttt t 511

<210> 143
<211> 463
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(463)
<223> n = A,T,C or G

<400> 143
actgcagtga ctcatcagag tagaaggagt attcaataag tgggacttct gtgtcgttaa 60
attgggcata tgctaaaaaa gtgccgtttg gagaccacca cagagcagag taggcactga 120
agacttcctc ttcataaacc cagtcagtta ttccattata tattatatct tctttccccg 180
tccatgtgat tctgtaactt ggtaaatttg gttcaatttt aacataaatg tcattgttcc 240
aaacatatgc caatttatga cccactggtg accatgtgac ccactgtgtg ttgtttggaa 300
tcctctcttc tgtaatcagc tgccttttat ttaaatcata aatgtcatat gaagctgtgt 360

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aggaatgcct ccattgcttc acgtagttgt attctaagag aataaactgc ccatcangag    420
atattgaata atcattgata gaatgnccaa actcatcaaa tgt                      463

<210> 144
<211> 297
<212> DNA
<213> Homo sapien

<400> 144
actcattaat attattttgt tttgagaaag ccagaaatga ttctaagaaa taaacaataa     60
taataaaaga tgtaattaat atactgtatc cctttttaagc caaagcacac tttttacctc   120
aagactgttc tgacttttac attcttaatt tcctttgtcc aaaataggac cccattttaa    180
atagagttca tttgaattga gttcataatc taaagtcact tttccccaca agatgttttc    240
atttcagtat ataaactgct aagcggcaaa tgactaagtc agttataaag aatttgt       297

<210> 145
<211> 356
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(356)
<223> n = A,T,C or G

<400> 145
actnctgcac ctccttcagn aggaggncaa aggggaatgg cgacagctgc tcaatccttg     60
tgatggncac ctgccccacc atgtcgcgtg ctttgcgctc ccgggttgag gtcataatac    120
actttgccgg tgcagaanag aagccttttg acattttctg ggntctgagc tgcaaggcca    180
tcttctggga tcacccgctg gaannggtn cctggaagca tctcatcaaa gctggatctg     240
gcctcggggn ggcncaacan ggatttgggg gtgaagataa ttaacngctt ccggaatggc    300
agcnggatct ggcgtcgtaa cacgtggaag aagctgccac gagnggagca nttgac        356

<210> 146
<211> 355
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(355)
<223> n = A,T,C or G

<400> 146
acagttttgt ttctcgtaa ggggagcatc atagggttac tttataccag ttgtaacatt      60
ttcattgttt ttggttgttc ttttttcttt ttttaatggc agctaaagat atacagatta    120
ctgttaaatt gcagtccttt ttttttaaa natattttct tgagttattt aaaacatggt     180
aagcctggta ttttttaatc aaacaaaata tttatgaaan gggttttctc ttaattctgg    240
attcatcatg gctttctaat accaattgta atatttacaa tattcaccaa aacttagaat    300
tttgcaaatg ctggaattct gccagtgttt ctttgctaag ccttgcatgc aaaat         355

<210> 147
<211> 209
<212> DNA
<213> Homo sapien

<400> 147
attttttact ttatatatga aaatgtcatg aaatttataa gcaataatgt attgatactc     60
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aaatttttaa aaatttttaa attttaaaat atttaatcaa cttctattat ttttcctctt 120
ctgggatgaa ttaagtggca aacttggcca ttctaatatt tactcactga tagccaaatt 180
ttatagcgtc tctatctaaa gaagacagt 209

<210> 148
<211> 445
<212> DNA
<213> Homo sapien

<400> 148
actcccagca aatcctctga atactccaca gactatgtta cccagtccca aggctattaa 60
ctcctgattg ccatcaagtg gataatcgta tttgagggaa tagacgctgg caactgaaaa 120
ggccactgca aatgcaacca ttgcgatgcc gaagcaatct cctacggtgt tttggaaagt 180
ctccacgtca ggtgtaatag gggctgaaa tccaggattc atgtccccaa ccacagccac 240
tttaaacctg tttttaaagt cacagccgta ggatacacct gctgcaatca cggtcataat 300
gaattcgatt ggaatgggca ctggaagttt gtctttgaag cgctgattta tttctttaac 360
aatggataca accaaaagga caatcagagc tgtcaccagg tctgcaatat tagtcttctc 420
tatttgtgag aatacagagt atagt 445

<210> 149
<211> 585
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(585)
<223> n = A,T,C or G

<400> 149
actattaatg agaacgaaat acacattagg aaaatggagc catttcaatc tagtggtttg 60
ggcaagatgg ggaagagaag gggaaacatt ctagtttctg gattacatta ttatgcccct 120
cctgaaaagg tggttgtcat ttgcatttat ttaaagcagg taatatgcag gaatgtaact 180
gaggattatc ttcaggcaat cagcaagata tcctcctcat ggtcccttta gctctcaaaa 240
gcaatgaaat cctcctgttc tcatttttac tgctgtggtt gtgctgctga acaatactat 300
cttctcaaat tccatgccac aaattcagca ataacttttt ggattgaatt tagcaactac 360
tgtaattgga tgctgatgtg gacaaaatat attgatttcg atttcactcc cgaatgtgat 420
tgccaccagc tctttatatt gctgctgtgg tattttaaac cagaagcttc tttaaattat 480
gttgcaaact gatctttgnt tttatgtttt ggtttggttt tatttctaag tgataagttt 540
gaaacacaca gctttaaatg attttttat tgtgggattt tgggt 585

<210> 150
<211> 508
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(508)
<223> n = A,T,C or G

<400> 150
acaatgtctt agaaagtctt taagtcacat accatgaatt tttgcttcat tactgaccat 60
atatgacctt ggaggaactc ttttttttt ccttctactc atttctgttt ccacctaccc 120
tgactcaccg tatttccagt cttctacccc tgcagttatc ctagtccagc aaagtcattt 180
ntttcaaaan anacatcatg tctgaaaata attactggta gtctaatatg agccanagta 240
aacagctcct catggtcaat gaacatgttc aggaagcgat caccttgatg cttgaaccca 300
accccanaca gnggacaatt ntactttgaa atatccgnga atatttactg ggggatccaa 360

tttaaacttc tttnttctnt agcctttaaa ttacacaact ttgaactgac acggatctnt 420
tacaaanaac aatgcggcac tgaaggaana gatgattcct ttactcaaac ctgcaggaat 480
cagcctatta acaggcaggg gaaacggt 508

<210> 151
<211> 434
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(434)
<223> n = A,T,C or G

<400> 151
accatgaata aaagtgcatt tcaataccag ttttaacaac agcatatagg gcagacataa 60
aagaagacca cttccgaaac tagtgcaaga gattgagcat taggcacaaa gggagaaaaa 120
tgaaaagaat gaacttttg aaggaataag cattaagact agatgaccac attattatag 180
agacaaagct agcagcaaaa ttttaatcct tgatgatgta gctttcaaaa tttgcattct 240
ctcctatagt ctaccctata cgaacagctc ttcctatttt cctctttccg actgtgaagt 300
tactaaaatc ctaacactaa ttccatatat tctgtgtgcc aggcatttcc catgcttgct 360
atctaactcc cgggtaagca aatcttgnag taagaggcag tacctgcctg gcggccggtc 420
aagggcgaat tctg 434

<210> 152
<211> 320
<212> DNA
<213> Homo sapien

<400> 152
actttgcaat catctttcct tttttcacat tggtaaaaat aagtggcatc cataggatca 60
tgatttttaa tttgttgcct ctgaagattt cactccatca agatctgcca atcttcaata 120
ttctggctaa atcttggtat gtggttttta aacagtcact ccgtttcaaa gtctgtcttt 180
ccttatagaa tgtggaaatt atttctccat accttgtgat tttgacctga gtgctaagag 240
aatcactctc cttacctagt tatctacaaa tgttcattcc agaaatgttt agttactgaa 300
ttgaatgaag acatctcagt 320

<210> 153
<211> 459
<212> DNA
<213> Homo sapien

<400> 153
acctcatttt tattagccat tatcttcatg ctggattcta atattctttt taatggtgat 60
ctgttcaatg acagaaactt atagagagaa aattccttct caatttataa acaaaaattt 120
taaaagcagc atttttgatg tggtaggaag atatttatga caaaagcagc tactgcccta 180
aactggcaaa aacaacaaaa gaacaaattg ttatttaacc tttaaataac gagtctctat 240
ttgctataaa tctacaaata ttttaaatat atttcctcct actgcaataa aaattaagat 300
aactctctgt ttaacagctt ttgaagagtt aattttataa ggaaataaaa aagattgact 360
tgcctcctga atgtccagtg ataaactgaa ccctaatttc cctacctcaa caacataaaa 420
atgatgtaaa gtggatcaaa gtatgtaaca agttaatat 459

<210> 154
<211> 503
<212> DNA
<213> Homo sapien

<400> 154

acacagcctt gttgccatgt ctgttgtggg ccacaatcgc cttgtccttc tgaattatga 60
tttctggaaa ctcctgggcc aggtgagtca cttgaatggt gcacttaatg tggagctgag 120
ctccttccat gatcattccg gtggggctga tgtggaactt gggtgtagag aaggattccg 180
tcacggtgac cagttcactc ttggtagatt ctgaggtctg catatggatc ccagaaatga 240
tcctagcttg acgtcggaag gataaaacgc ggtcctgttc ctcaacgggg aattccagta 300
tcacaaaatt ctggtctcga gaattcttct ctcttttcag cttgaccatt ttttcattta 360
gttcaagttt ttcaattgtg aagtgtattg gggccttttc ctctgggaca gaacagttga 420
ccctcacgat cccaccttgg atggcctctt tcttgtccag tgtcaccctg ggactgggca 480
ctcctttcac caacacctgg tac 503

<210> 155
<211> 364
<212> DNA
<213> Homo sapien

<400> 155
actaaatata gaacacttaa caaatgccaa tcttttgctg agtgaaaatt taacaattta 60
ctgagagaaa agtaaatata agaatttaaa gttcctttca tacttgatca tactataagc 120
attgccatca tttcaatgca catatatttt taaaaaacaa ttttctctct caaactcata 180
ttaaataact ggattttaaa acatttcccc catccacaca aaaaagatat gtgggttcta 240
attattcttt gctatttaat aatgctacct ttgaagattt ctacataata taaacattcc 300
aattctgaag caaagtattt cagcattttt caaaagtctc taatatatct tttgtttgta 360
gcgt 364

<210> 156
<211> 452
<212> DNA
<213> Homo sapien

<400> 156
acatatatgt atattatacc aatagctagt aatttcaaaa aaaacattga cttgagtgtt 60
agataaccat tctctaaatt cagtttttga tgtttcaaga aacccaaaag cctgtctttt 120
cacctacaga ccctttgtgc acgtggcaaa tcacctctga aaggcaaaaa actaactgga 180
ttctcttcat ttgttcaaaa aagagaagaa agctttaaag atatgcctat aaataaaaga 240
aaattaggtt gctatattat gattgtgcaa taagtattaa tttcattgaa gtttgaccct 300
gttccatgta ttagatgact aagacattta actcttaggg atgttgaaag cgcaccacaa 360
aacataagta atcaataaag taatgtttga agacttttag tatatactgc ttattcaggt 420
aattaattat tttgtaaata ctaatagcat at 452

<210> 157
<211> 224
<212> DNA
<213> Homo sapien

<400> 157
acatgaacag caggctgttg cattgtaact tgtggctgtg cattaagatg ttgctgagga 60
ttgcgaactc ctgcagcata tttatactgt ggaacggtgc ggacagcagg agtagctgca 120
gcggctgcag ctgcaggacg tggacccatt gtctgtgttg atgtgttagc aacacgctgt 180
gttgacatga ctcgtggaac ctgtgaagaa gctggtctca tagt 224

<210> 158
<211> 623
<212> DNA
<213> Homo sapien

<400> 158
acacatttca ttatgctgcc ttttctctta tgattaaaac tttagccctc attcgaggtt 60
tccaatggtt acttttagtg gaggagttcc ctagctttta aaaaaccact tttcctctaa 120

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gattccatta tttattgaaa gaagtctttc tagaaatgtt aaggaggatt ttaaatgaac    180
acattcaatt aaaaaaaaaa tcacgtattg aacatctacc aagcatctgg actcttcgga    240
acctagtaaa atgaaaaaat ccagttttaa caacagtaac ttcattctgc gggtatacag    300
agacaagcac gtttcttctt ttggtctaat ttattctaaa cgaagaagct gggaactgac    360
aaaacaggac aggttgtttt taatccagtc tacaaataaa caagacaatg cctgagttag    420
ccctctatat agatttaggc ttatgctgac ctcgttgtaa aatctgtatt taactaaaag    480
ttaataaaaa tacatatgtt cattttaaaa taattactga ttttgcttgg ctatcccacc    540
ccttaccccc aaactcatat atttttagga caagattttc ctgcataacc acaacctgtc    600
tcctcccccc cacccccatc ata                                            623

<210> 159
<211> 422
<212> DNA
<213> Homo sapien

<400> 159
aggtaccatc ttcttcagaa ctgcatctaa gaggctgtgc tggctgggaa tcatacagct     60
gtgggcaaca actgcatcag ccccaaggct tccctccaga ccaaaaggtg attcatggcc    120
cctggttaat atcaccctag gttctcccct gtcccagttt taacataata tttcatagaa    180
atactagtgc cataaaaagt caacatttca aatataaaaa ttattttata caaatgtaat    240
tcataatcat tcttttaaaa tacagcattg ttatatatgt ttgaaacatt attaaaataa    300
atatttccta gagaaaaaat tttgcttcac aaaattataa aacagaagca tataaaacta    360
attcatgatt ggtgcttctt cagtgtgtct ctcattctct cttagtgtag acagcatgaa    420
gt                                                                   422

<210> 160
<211> 393
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(393)
<223> n = A,T,C or G

<400> 160
agctcactct tttatctgtg tggctgattt cattactgtt tgtgatttgg agctactcac     60
tggatggtga cctcttttca ctttctctac tccatgtctg ggcatgaccc agctttggac    120
tccttgagcc cctctctaat ttaaatttga tattattaat tatccaggta attgtcttcc    180
gtgtggttgc ctccttcccc actccagtat ccactttcag caaaacgtct tgcttcaagt    240
cccagataga agagtctttg acttttcttc agaggcttat tttagctaga atgtttaaag    300
ctacagatgc ctatctgctc atctttccag ctggattagg tgttgcttag atttgctagt    360
tgctttaagt attacacagt ttttgnattt atg                                 393

<210> 161
<211> 223
<212> DNA
<213> Homo sapien

<400> 161
accacttaat tactggcact gagtatcact gaatttctta gttttctagt ggggaaacat     60
tattgagaag ccctccctta ttttaagtaa gttgattaaa tcttatgtga gttgccagtt    120
gtaattttc aaaggaaaaa ttttgatggg gtggaggaat gaattgccag ataatctttc    180
tggaattccg agagaattcc aaagagggtt ttttttttt tag                       223

<210> 162
<211> 487
<212> DNA
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<213> Homo sapien

<400> 162
acaagtctac attcccacta acagtgttta aacgttcctg cctctgcatt ctcgtcagca 60
tttgttactg tcttttggta actgtcattc taacgggggt aagacaatct ctcattgtgg 120
ttttgattct ctttagaacg aatatttctc ctcattcctc tactcttaat aatggatttt 180
ctgaaaaaca tctattaatt ttatgcacta ttcaattcaa acaacttttt aaaagttgcc 240
aaatctgtca caaaatatta aacaacaaga aaaatatcta aaggtaaact tgagaggggt 300
gtaaaacaaa agactctgag agcgcactta gctgtaaaac aatcattcct attcctaaat 360
tgagtgtttt tggttacatg ttctaagtgc cttacaataa accaggcaat gtgctttatc 420
tggagaaagg gagccctaac ttcaaagttt gagttcctcc aactttttta atagttaaat 480
ttcaagt 487

<210> 163
<211> 500
<212> DNA
<213> Homo sapien

<400> 163
acactggatg cagccatgca tggatggttt ttctttattt ttcagtgatt tcctctgaag 60
cagctgcact gatacatttg ggagttggtg gcttgacttt gtccataagg ggcgtggcca 120
cttcacatga tggcgggcct ttaagagcac aaagaagttt aatatggaca acaacaggaa 180
aaagcaagaa gaaaacaagt agggaaaaac agctaacctg gagagaaaga atttctttaa 240
cctttatgtt cttcattaaa aatcttatct tggactgatt tgagggattt ttagaaacat 300
ggccttattt tatataagca ttaccttccc aggaatcttt gttgtatatt aatttttgat 360
aaccatttga ttaactttaa aattaagtat atgtgtgtat atatacatat gtatgtttat 420
atacacacat gtatctgtat agttttatat atacatatat acacatagac atacagagaa 480
ccactacttt gtaatagtgt 500

<210> 164
<211> 547
<212> DNA
<213> Homo sapien

<400> 164
actgtaatgg gtttggccaa atatcatctt tgatgacctc tcctaactca tcagcacctg 60
catcagaatg gtcagtaaac caggtaaaga agctctctgg ttcctcatgc tgcctcttcc 120
tgctggcttt attctgcgtt tgactcgaac gtttcgtcaa atcctttcca gatttccatt 180
tgatttcggt ggacttcgaa gatggatcac cactctcatt cagatgaaat tctttggaga 240
gaactttatt ttcaaagtaa ggattttcat caaaataaaa atctattctg taacctgatt 300
taatatcttc aaattctgtc acttcaactc tggtcaaata atgcagtgcc tcttcatctt 360
cctccccaag cagtgcagac acttgtggat ggttgacaaa tgttgttacc caaaaatttg 420
ggattttggc gatcaattct gacctcttct gaaaaaatgg ttggcggagt ttgttatatt 480
tctgttctac tttcaaaatc tcctcactgg cttgttcatt aagtctgtct atttcatttt 540
gtacctg 547

<210> 165
<211> 400
<212> DNA
<213> Homo sapien

<400> 165
acaaaactta caaagaagtc aaaagtctta acactcccat tctccaggaa ctcttgtctg 60
tgtcatctgg taggagggag gaatcctggt tccctcaggt ccttgtcatg ttagcttttt 120
gatagcttca atccactcgg ctcgctcggc cttgctgctg gcctgaatgt aatagtgtgt 180
gtcatcctta gtaatcactt tgaagaggtt tccctggaca ttccctttaa ccccagtggg 240
aacgccatta tcttccagag cagacacgag tgaaccacga agagaaaacc cacccactgg 300
cctgttctct tctttggaag ggtcatagta atgcaggaaa gctggatcct tccttagaac 360

aaagcgacgc accttccagt ttttcctctt gtgccctgct 400

<210> 166
<211> 274
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(274)
<223> n = A,T,C or G

<400> 166
ggtaccttca tataataaag ttaacaaaaa taataaaata ttaaaaaaaa gagccagctg 60
gcactgccaa ccaattccta tagtagcctt agaaatccta atcctgtaga atttcctctt 120
gtagtcaata agcaccaccn tcttcaggag tatttcagtg tattgttatc tacaccaagc 180
aagcctggtg atgcagctac ctgagttctc ttggttatgg gtgaatgtta tcttcattca 240
taacttcccn gctttcatgt aggtggggat agag 274

<210> 167
<211> 478
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(478)
<223> n = A,T,C or G

<400> 167
ctttttaaaa tccaatatat tctgccaaga atatgccttg atagttagcc ctcagcccat 60
aggtgttttt tgttttttaa cagaattata tatgtctggg ggtgaaaaaa cccttgcatt 120
ccaaaggtcc atactggtta cttggtttca ttgccaccac ttagtggatg ttcagtttag 180
aaccattttg tctgctccct ctggaagcct tgcgcagagc ttactttgta attgttggag 240
aataactgct gaatttttag ctgctttgag ttgattcgca ccactgcacc acaactcaat 300
atgaaaacta tttaacttat ttattatctt gngaaaagna tacaatgaaa attttgntca 360
tactgnattt atcaagtatg atgaaaagca ataganatat attcttttat tatggtaaaa 420
tatgantgnc attattaatc ggccaaatgg ggagnggatg ntcttttcca gnaatata 478

<210> 168
<211> 213
<212> DNA
<213> Homo sapien

<400> 168
acaaatgtaa cagtaatgat aaattctctt ttccaaggga aagagaaacg ctgcagaatg 60
gacattaaac aaggcattat gccctacaag caagacataa aatgtctaag ggaaacttca 120
gcataaaaat gttgaacaca taatgtgaga taatttgaat aaataacaac tgacattctt 180
tttttaaaaa aaaagtataa aaaatagatg tgt 213

<210> 169
<211> 341
<212> DNA
<213> Homo sapien

<400> 169
actggctgcg aggcgccagt cgatcaatgt atgacaggag ctgagacttg gccacaccag 60
gatcccccat cagacagatg ttgatgttgc cccggatttt catgcctcga ggagactggt 120

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ccacaccccc gactagcagg agcagcagtg ccttcttcac atcttcatgc ccgtatattt    180
ctggggcgat tgaagctgcc agcttttcgt agaaatcctc ctctgcaatt tgcctcagct    240
cctccctggt gagctctcca gccccagact catcatcctc actcttgttc atcttcacaa    300
tccgatgggc ttccaggtag gtttctgaga gtaaaccctg t                        341

<210> 170
<211> 543
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(543)
<223> n = A,T,C or G

<400> 170
accaatgatc atgcttccat ttttttagt tttaaaccac caaaccaata tttttccttt      60
aaattttaat cttataatat agaaatctta tgtaaatgaa attttgtcat gtttcaaata    120
aagagaactg aagtagaaaa tagaaatgcc agtaaacaac ataatgttta atttacaact    180
tacattaggg gtttgggga atgctaatta tatattgaga atatacatta gaactcttca     240
aaatgggctc ttctaatgag gtcactactg aacaaaattg ttccctcttc tgttaaatag    300
aataggttta aatgactagt caaatgaatt attttcttct tgttaaataa attaaatctt    360
actttctttt aatgaccaac cttaggtaaa acaaaaatat tgtaatccta gaaattatcc    420
tccagctttc tcacctgaaa atctattgaa gtgatccctg gtcatcctaa taatgggatg    480
agggaagttt ccagcagatt tcaggctgnt cttaaaggtt ttggtggnca ttttctcaat    540
agt                                                                  543

<210> 171
<211> 280
<212> DNA
<213> Homo sapien

<400> 171
acatactaaa aatatttaaa atagagaata ttcctcacag aggacttttt tctttaatta     60
ctactaaaaa aataattaca aagtccaaac aggcagagag atttagcaca ctgatcacac    120
gattctccat catcctccac gcttgctctg aagagggttt aaaaagtcca gtttctcgtt    180
gatttcgctg ctccatttag ccaaggttgg cctggccact gattggcaca agtgggtaat    240
gcgcttggat aggtcatgtt tgtgtcttgg aaatttgggt                         280

<210> 172
<211> 463
<212> DNA
<213> Homo sapien

<400> 172
caggtactat ttaccctatt aataagttcg gtctctgctt gcaatctttc cattgctcca     60
gcataccagg gttggcaaga ataatctact ggtttgggca cacatgggca aggcttgact    120
gcatcacttg gaaaaaatcc aacctctcca gatgctaaat ttctgccctg ccaaaacaga    180
ctgtgtgcat ctcctttcag aagttcaacg gtatccccgg cctggagctg taaagggggt    240
ccttcatgca gagctggggg tggtgttcca gaatagttcc taatgacctg catctttggt    300
aaacctggat ccacctgttt aggagttctt cgcagtccat tggtccgttt ctctggtagt    360
ttgagtgtcc cttgttctga aagaaatgta aaaattggca ttgtcagtgt aaagttattt    420
tgtttggtta gcaaccttag ctttctctgc agagtggtaa aac                      463

<210> 173
<211> 165
<212> DNA
<213> Homo sapien
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<400> 173
acccaaagaa ctggtggcct caggccacaa aaaggaaacc caaaagggaa agagaaagtg 60
agaagaaact gaagatggac tctattatgt gaagtagtaa tgttcagaaa ctgattattt 120
ggatcagaaa ccattgaaac tgcttcaaga attgtatctt taagt 165

<210> 174
<211> 532
<212> DNA
<213> Homo sapien

<400> 174
actccatctc tttgactgaa taggtcattg atcctatcaa gggataacaa tgtttttgcc 60
actggatgtt gatgttccta tccaaatcca cagcaagctg gtgttgcaat tttccagatt 120
catgcagatc cactgacttc agtgtgttga tactggcttt gaagtattcc atccactggc 180
ggatcgtgga atctcccatt aggtatatga gttttcctct caggcattcc ttcattttga 240
ctgtagccaa actacaggag acaggattcc atgtgtttct ccagacatgc ccactgggga 300
ttgtggatgt cattccaaac ttgcatttct ctttcattgc aactgtttct ttgttgcatt 360
tggagacact aattgtattg aatttttcca taatctctac acccacattt gacctttcaa 420
agaggctctt ttcttgtttg ctaagataag aaactttctt gttcttagaa tacatgtgag 480
tgagtgcagc acagggcatg tgttgaggcc tcacacagta gaagccttct tg 532

<210> 175
<211> 374
<212> DNA
<213> Homo sapien

<400> 175
taatcacctg actgagctcc aattaactga ggagaaacgg ggtggaggag agggctggtt 60
gctattcaga cttgataatg agattgatct gtcccatgga gagtgaaagt tcagttccac 120
ttctgcctcc ttctttccat gctgtcctca tgctctttat cctcacttcc tcagtccctt 180
caacactcaa aatctgattt tatttctctc tcacacgtat caggggcagt ttctgaagtt 240
gctgaggttg aattttcttc acaaacctct ataaaacatc agcagagaac atataaatac 300
attttgatta gcatacattg caaatttctt cccacaatgt caggggatga aagcaggtgg 360
tccccactga gagt 374

<210> 176
<211> 428
<212> DNA
<213> Homo sapien

<400> 176
actgcaactg ccagaacttg gtattgtagc tgctgcccgc tgactagcag ctggactgat 60
tttgaataaa aatgaaagca ttaaagggtt tccctacaaa acatttttct ttaaaatact 120
tttgaaatgg ctataagcag ttgactttca cccttggaga gcatcacact gtgtgaggtt 180
cagtgattgt tgaccctccc cagcccctcc tgcttcttta agttatctgt gtgcgtgcgc 240
ttcctctcaa tcttctttgc acgctcattt ctttttctct gacccatgag aaaggaaaac 300
ttactgatga taatttttaa atagtgtaat ttattcattt atagcatgtc aggataaatt 360
aaaagaacat ttgtctggaa atgctgccgg gagcctattg tgtaaatgta ggtattttgt 420
aaaataac 428

<210> 177
<211> 318
<212> DNA
<213> Homo sapien

<400> 177
acctgaacga agtcgcgggc aagcatggcg tgggccgtat tgacatcgtg gagaaccgct 60

tcattggaat gaagtcccga ggtatctacg agaccccagc aggcaccatc ctttaccatg 120
ctcatttaga catcgaggcc ttcaccatgg accgggaagt gcacaaaatc aaacaaggcc 180
tgggcttgaa atttgctgag ctggtgtata ccggtttctg gcacagccct gagtgtgaat 240
ttgtccgcca ctacatcgcc aagtcccagg agcgagtgga agggaaagtg caggtgtccg 300
tcctcagggg ccaggtgt 318

<210> 178
<211> 431
<212> DNA
<213> Homo sapien

<400> 178
acttgaggct tttttgtttt aattgagaaa agactttgca attttttttt aggatgagcc 60
tctcctagac ttgacctaga atattacata ttcctccagt aagtaatact gaagagcaaa 120
agagaggcag gattggggtc acagccgctt cttcagcatg gaccaagtgg gccttgggga 180
ttgcagcgtt ctcgaagtgg ctgtaggact cgaatttaca gaaagccaca gaggtgcaac 240
ttgaggctct gctagcaagc caccagtgag gctattgggt aaccaccttt ctatacagga 300
gattggaatc tactttgtca tttatccacc acagtgacaa aggaaaagtg gtgccgttat 360
gcaatccatt taactcataa acatattact ctgagtaact ggccagccat tcatcggatc 420
cttcattggg t 431

<210> 179
<211> 323
<212> DNA
<213> Homo sapien

<400> 179
actgcccact tttacacaag ctgcagcaga actcagttct actgcaggtg agagtattgc 60
accatcatta acataataag gacctcagaa tccaaccttg ccaaagaatt caactcctag 120
gctcagatta atggaagtgc tgggcacatg ccacctcctg ccattgtcac agttcagctg 180
tgctggcccc gacacagctc cagttccacc catgacatct ggctgaggag gcttatggga 240
gcggcttctc atgcacagtt actgtccctc tctggagggt cctttaatgg ggactgtgca 300
aagcagtgac actaactgcc agt 323

<210> 180
<211> 409
<212> DNA
<213> Homo sapien

<400> 180
actgtgttcc tttgcatgtt tcttctttaa agaatttagc tccttctgct gtttctttaa 60
atgcttcaag taagccttca tctgctttaa gtcttctatc cttacttgag ggataagttc 120
aatacctttc ttggcttcca caccagaggc cagggcagcc gtggtggttg gtctgagctc 180
agagctactc tgaggggtca catttgcttt ggcggtgttg gcctttcctt tcttgtcatt 240
tttggaagtg tcactgggca cgtcggctat gtcactagtt tcaatgccca tagctctcat 300
ttggtctgct ctcttttctg taattgagag aaatttcttt ggatctgata aagcatccac 360
gatatctcca aatccatcag gcacatatgt tttaagaaca atattgcaa 409

<210> 181
<211> 460
<212> DNA
<213> Homo sapien

<400> 181
acaaagattg gtagctttta tattttttta aaaatgctat actaagagaa aaaacaaaag 60
accacaacaa tattccaaat tataggttga gagaatgtaa ctatgaagaa agtattctaa 120
ccaactaaaa aaaatattga aaccactttt gattgaagca aaatgaataa tgctagattt 180
aaaaacagtg tgaaatcaca ctttggtctg taaacatatt tagctttgct tttcattcag 240

atgtatacat aaacttattt aaaatgtcat ttaagtgaac cattccaagg cataataaaa 300
aaagaggtag caaatgaaaa ttaaagcatt tattttggta gttcttcaat aatgatgcga 360
gaaactgaat tccatccagt agaagcatct ccttttgggt aatctgaaca aggccaaccc 420
agatagcaac atccctaatc cagcaccaat tccttccaaa 460

<210> 182
<211> 232
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(232)
<223> n = A,T,C or G

<400> 182
actgacagat taatggcttg cctagagctg tgcaagaaac agcctgccag nctgtcattg 60
nnagggacca gggcaaaacc aagagctgtt cttcccagaa gagccctgca aacacattgg 120
ttcgtgcttc cctttacttc ttctggtcag ataccatgaa tgccagtcat cagtaaatct 180
taatacactt ttgctttatt ctcacatgcc attcaccaga ttatttgatg gt 232

<210> 183
<211> 383
<212> DNA
<213> Homo sapien

<400> 183
atgttattta aaagatgaaa tttcatggtt caaatgtatt tttctcccat aaaaatattt 60
tctcttccat ttaaatatat acctaatctt tgagaaatct tgcacaaatg gcattttatt 120
aaagaaaatc taatttacaa agctttgtaa attttgagaa aaacattcat agatcataaa 180
caaaaatttc aatatgcaat attcaaattt acaagaaaat aagcacaaac ttttagacag 240
tgcagttatt gctgcactcc tttaattcct tatccagagc ccaaaaaatg taggcaaacc 300
ctaaaaatgt agcagaagca tttccgcaca ctggtgtcca gaatctagtt tgtgcagaaa 360
tgtttccact agatttatag agt 383

<210> 184
<211> 444
<212> DNA
<213> Homo sapien

<400> 184
acagacacaa acatataaat atatgtatgc acatatttgt catacatttt caataaatga 60
tatctttatt attgtttaat gaccttttt ctcttgtgaa ttttgacata aagtatattt 120
tataaaataa gagagttgtt gacttacgat gtattttgta taatacaatt ttgatctctt 180
ctgctctcat ttggttgatg tttgcctaaa atgtcttctt ccacttgcca ctttcaggct 240
gatttcacta ctagatctca agtgactctt gaagagaggc aagttggatc ttggtatata 300
aaattttata taatccctct attcaatgta tgtgtattga ttggcaagtc tatttttaaa 360
atatttattt tctgaagaca aagattactg ttattttatt gtttaatgat tcttgtaggt 420
ctgtttctca ttctatcttc cttt 444

<210> 185
<211> 289
<212> DNA
<213> Homo sapien

<400> 185
acttgtgaca ggcagacgtg attgcagcca cgaacacgat gaactcactg aagtccacct 60
gggcatctcc attggcgtcc aggtccttga gcaatttatc cacggcatcc ctgtcttttc 120

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cactctgcag gaagcctggt agctccttct ccatcagcac cttgagctcc cccttggtca      180
gggtctgcgt gctgccctcg ctgcccgaat atcgggaaaa gacgtctatg atcatgccca      240
tgactgtctc tagttccgtc atggtgctag attcagaccc accttcctc                  289

<210> 186
<211> 407
<212> DNA
<213> Homo sapien

<400> 186
acagacaaaa tgctcaggat gccatgattg ccctagagca tggatcacct tcccagcaat       60
cggtttctgg caggatgcac aatggccctt gggcactgtg gcaatgccaa ggtcctgcaa      120
ttcctgctcc agacccccaa gcattgagtc cagggaggcc ttgtgatcct gcttgtctgg      180
taagtgcttc ttgccagcat ctgctctcac tgcaaccttg gcctgcatct cagtcaggtg      240
agccatgagc tcatccaact gagcagctgc tgacgtttta gaaggtggtg gtgattcctt      300
tggctcttgg gcttcactgt agacattgag ctcctggata ttggtagtat acacgagctg      360
cgccggcaag ggacttgtgt tatcctgaat agaaaggatc tccgaag                    407

<210> 187
<211> 441
<212> DNA
<213> Homo sapien

<400> 187
actgcaagac ccatcttccc tccagttaat acactcccag gatgggctgc agagggggag       60
actctgagag aagctggagg cccacaaaag tccactgacc ctctttctgt cccagaaatg      120
aataaaggac ccagttgtgc tttccttcca aaatcctcaa caaagttgtt tgtgctccaa      180
gaaaatgtgg gaataaaaaa atcatgtccc aggtcatctt tgtgtgtgtg cgggggaggt      240
ggatgggagg aaaaggcatg tattaataga tactgctgct ataaaatgac ataaatcata      300
gcccttgatc tgtttctgta aacaatgcca gcttcttcag gttattggca actaccccta      360
atatacctag cccagatcct ttcataaagt caagtgctat atttccaaaa taatcctatg      420
aaatcatgaa ggttgtgaag g                                                441

<210> 188
<211> 323
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(323)
<223> n = A,T,C or G

<400> 188
acttagaaaa cagtccctgt ccatcagcca gaaaaggtga ccatcacccc taaagtaatt       60
tccaaacttt agttcagtgg gaaagatatg ctggtagtgc atattcagng ntgattttca      120
gtgctagtaa ccacttttaa tgccagaaat atgtaacaat gataatgtaa cgtcaaagtg      180
gttactaaag attatagcct taacttttt atgnaaaaga taaaatccat tcctcctccc       240
agtgagcaag catggcttgc atttctcaaa aatgagaact tccatggcag ccaagaaaac      300
gtcttctcag aggaactttc gtt                                              323

<210> 189
<211> 225
<212> DNA
<213> Homo sapien

<400> 189
caggtactcc ctgatctttt cctcagtggc ttcaggattc agacccccaa cgaagatttt       60
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cttcaccggg tccttcttca tagccatggc ctttttaggg tcaatgacac ggccatccag 120
cctgtgctcc ttctggtcta ggaccttctc cacactggct gcatctttga acaggataaa 180
cccaaaccct cttgaccgtc cagtgttggg atccattttt attgt 225

<210> 190
<211> 501
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(501)
<223> n = A,T,C or G

<400> 190
acagctgaag ttngataaca aagaaatata tataagacaa aaatagacaa nagttaacaa 60
taaaaacaca actatctgtt gacataacat atggaaactt tttgtcagaa agctacatct 120
tcttaatctg attgtccaaa tcattaaaat atggatgatt cattgccatt ttgccagaaa 180
ttcgtttggc tggatcatac attaacattt tcnagagcaa atccaagcca ttttcatcca 240
agtttttgac atgggatgct aggcttcctg gnttccattt gggaaatgta ttcttatagn 300
cctgtaaaga ttccacttct ggccacactt cattattggg agtgcccaaa gctctgaaaa 360
tcctgaagag ttgatcaatt tctgaatccc catggaaaag tggtttctta gttgctagtt 420
cagcaaatat ggtgcctata ctccaaatgt caactggagt tgagtaacga gctgacccca 480
gcaatacttc tggagatctg t 501

<210> 191
<211> 436
<212> DNA
<213> Homo sapien

<400> 191
acagtgcatg gtgctgtcac ttggaaagcc tttcaatgtt gtcttcagat tgttgtgatg 60
aatatgaaac atgcagaccc tcctttataa agaaaaagac cttaaaactt gaatatgaga 120
taattttaca ttttaaaagt ttatttgatt ttcatattat tcactttcaa agccctttca 180
aatagaaaag gtatgaactt ttggggggat aatttatgta tcgtaaactt attagaacaa 240
aatattcctg atgtataatg agttgtttta tttatacaac tttttcaatg gtagtttgca 300
ctattcttta ttatgctaca ggtttattta ttatgaaaca aaggaatatg tattttatgt 360
attttaccat gcataggtta actctttgcc acagatttat tggttcttga tacacctaaa 420
ataaaaaaaa atgtgt 436

<210> 192
<211> 319
<212> DNA
<213> Homo sapien

<400> 192
ccagcgacag actttgcaaa catgcagatg gttctcacat gtcttccttg tctcattttc 60
agggcacgtg tcctaggttc tttcgattac gtctctcaag gcaaggtttc cagatctctc 120
tgtatcctta cgcttccctt ttggatgcac cttaatttta aaatacctct ttttctcatt 180
aattagatca cttcaagtta aatacaaaac atggcaagat ggatttaaat ttagagggat 240
ataagtatac ataagagaag accaatctct actttaaaa atgcagttaa ttaacaataa 300
agtaaaatat agtgaaggt 319

<210> 193
<211> 586
<212> DNA
<213> Homo sapien

<400> 193
acaagaggcc atttgtcttg cctttttctg acatgtgcat actataaaat cacaggtagc 60
caacatttag tatcagtaaa aaacaactac gtttgttcac ctgtttggca tagggagaaa 120
acaatgtatc tcatagcatt aaatgataca gccttaacac atatgatgct catatttgca 180
aagttcccaa atgttgagaa gttctagtga aaagtcatac tattgtgcaa agatgaaaat 240
ttggggccaa tgtctgtatt caaaataacc aaaatatatt ttaaagcaaa atatatcctg 300
atactactat agattctagg aattgtccta aaagagtaaa gtgttgtttc ctttctgaac 360
atgaataaca tcaaaggaag aacccagttc ttaagactta agtaggaaat ttatagaaat 420
ttgatttata ccagtagtaa taacattcat aaggaaaaac tattaggtaa caattttctc 480
caagaagagg atcagattac ttaaaattgt tggagaattc tggttgtttg cgcaataatc 540
atagtgattt acattgcttt tcttctttca gagcaataag aaagtt 586

<210> 194
<211> 214
<212> DNA
<213> Homo sapien

<400> 194
acattttat aactggaatg tttatgtgta gtgaagctct gagaggactt tgcattagat 60
ctcagcagca taatcagaag gttgtccttt gtctcagcaa tttttaagct aatagtagca 120
gaaattgcag tggaaataga ctgctttgcc acaacattca gaaaatcatt tatcttttta 180
ttgcagttct tgtcaccaaa caatacattt tagt 214

<210> 195
<211> 325
<212> DNA
<213> Homo sapien

<400> 195
actgtacata tttgcaatca cattgtgcat agattcttaa tggtagatat gatttctttt 60
gtcaggctac aacaatgaac tgcagattcc ttgtttgtaa tgtaaatgat tgaatacatt 120
ttgttaatat gtttttattc ctatgttttg ctattaaaaa ttttataaca tttccaagac 180
aaaaattcca agtttatgct ttgaagaatt tatgtaatta aaatttcact aaactaatct 240
ttttagttta ggaattattt gggttttgac actggaagtt gcgccaaata agcatcagaa 300
ataggagatg cttaacattg ctata 325

<210> 196
<211> 382
<212> DNA
<213> Homo sapien

<400> 196
actccttccc agttttttct ttatactgag ccttcaggga cagtaagcat tctacagctt 60
catttatttt agccttaggg gatttttcag cttttagctt acgaaccacc tccccttgtg 120
cagcaacttc atcatacaga gatttacttt ccagaatact tgctgaggaa ttagaagaaa 180
tattctgtcc tatttcagca ggagggtttc caggtttata ttcctggcca gttttctcct 240
tatattcagc tttcaaagac aaaagctgtt ttacagctgc atctacatct tcctttggtg 300
ctttcttggc ttttaattca cgaaccacat ctccttgaac agccactcta ttgtaaagga 360
ccaaggaatc ctcagatgta gt 382

<210> 197
<211> 648
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(648)

<223> n = A,T,C or G

<400> 197
acatccacat gttcctccaa atgacgtttg gggtcctgct tgccaacatt ctttattgcc 60
agctgttcag gtgtcatctt atcttcttct tctacagcct tattgtaatt cttggctaat 120
tccaacatct cttttaccac tgattcattg tgtttacaat gttcactgta gtcctgaagt 180
gtcaaacctt ccatccaact cttcttatgc aaatttagca acatcttctg ttccagttca 240
tttttccgat agttaatagt aatggagtaa taatgtctgt ttagtccatg aattaatgcc 300
tggatagatg gcttgtttaa gtgacccaga ttcgaagttg tttgtcttgg ttcatgtcct 360
aagaccatca tattagcatt gatcaatctg aaggcatcaa taacaacctt tccttttaca 420
ctctgaatgg gatccacaac cactgccaca gctctctccg acaaggcttc aaagctctgc 480
tgagtgttga tatccacacc agaaagccaa caaccaaagc cagggtgact gtgataccaa 540
ccaacaacca tctccggcct tcctgtctgc ttcaacatat ccaacatttt aacttggaac 600
actggatcaa ctgccttcac actgacacct ggtnctgatg nggcatag 648

<210> 198
<211> 546
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(546)
<223> n = A,T,C or G

<400> 198
acaatacagc accactactg agaagggctc gaggttttgc aatccaaggt tctgacttaa 60
agcaaaaata cacggcatag attgcaacag caaagaagtg tccaattaaa actagagggt 120
taggagacaa tacagaaagc agcccaacag gacccgcaac acattcgcca ccaagtttga 180
aataaagaaa acaggctttt cttagttgat gcagggaatc atctgtggca gaaaataatt 240
cataaagagc ctgagcaagg atattcacga caaaggaatg agatgttttt cttgcccagt 300
aaaatgattt tttggcctcg aaaatagctg catcatcata aaggtcaggg ataccctttta 360
gcagttttct ccatagtttt atatctttaa aagcaacagt cattcctcca ccagtaagtg 420
gatgcctcat attatatgcg tctcccaaaa gaagaacacc tcgtttcttc actgatgaag 480
gaggaaggaa gcttgctgca tggacctcag atgagaattg cagtggttct aagaatggtc 540
ntttca 546

<210> 199
<211> 275
<212> DNA
<213> Homo sapien

<400> 199
actatgtgta actttggcaa caggttgcag tcagccaggg tgagctcgtt gccatccaaa 60
aacttcctct gagagacacc ttcatcttca gcactggttt catccacttc ttctgggagg 120
ggggatgtta agtaattgtc taaaaccttc agggctttca ggagtccctt ctccagattg 180
tcattgagtg ctgggtttga attcttgatg taggcagaaa atttggcaaa tatgtccagc 240
ccagctgtgt tggactcagg gttcagagct gccag 275

<210> 200
<211> 423
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(423)
<223> n = A,T,C or G

<400> 200
cctgagaaat tctnaaaagt acgatgataa ggttgcaaaa atgaagaagc tcatcatact 60
aaaactagga aacatacnga tccataacan gacatgcnaa gcaaagttcc caaagtcaca 120
gacaagaaga gaatctcaaa tgctggaaaa tacataatta tggttgcatg atntaaccag 180
tgactctttc aacataaacc ttgcaggcca gaaggaaatt gcgtgctata gttgaggtgc 240
caagcgaaaa atagcttcta tgtaagaata acataaccag caaaactgtg ctacaaaaat 300
gaagaaaaag caaagacctc taaagataac caaacgtgga aaaattatat caacactaca 360
tgtgccatac aaaaaatgct gagaagagtc ctcctattaa aactatatga tgctaaaaaa 420
caa 423

<210> 201
<211> 560
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(560)
<223> n = A,T,C or G

<400> 201
acaatcgagt attttagaaa ttacatgaaa catgaaacag tttttgcaat tttttttaaa 60
ctgggcatct ggtttctaaa aatttatttg aaacaatcta gaattttctt ggtgcaaagt 120
gtatcatgtg gaatatcctc atatttttac catattttaa gaactttaag acgattaatt 180
gtaaataatt tatttgattg gtgcagttct aatccctaaa tcataatctt aaaatcagga 240
atgtgtggag aacagagcca tgtcatatca ctttgctctt accattcctt ttgatcagcc 300
tcaattcagc ctcattgtgt agtatgtttt ttctttctat gaaaaacaac agaaagcatt 360
tcattttatt tgcctatgtt caaatatgtt taataatgac caaagtgcat tctgagtttt 420
ttcaaggaat gtaatactgg agctttaaga acatacttag tttctcatgt gaaacttan 480
gctttgtctg angttttcct tcctctattg nctaatggtg aggtggtttt aggaattatg 540
ttttataact tttcaatata 560

<210> 202
<211> 366
<212> DNA
<213> Homo sapien

<400> 202
acgagcccca cagagcagga agccgatgtg actgcatcat atatttaaca atgacaagat 60
gttccggcgt ttatttctgc gttgggtttt cccttgcctt atgggctgaa gtgttctcta 120
gaatccagca ggtcacactg ggggcttcag gtgacgattt agctgtggct ccctcctcct 180
gtcctccccc gcacccctc ccttctggga aacaagaaga gtaaacagga aacctacttt 240
ttatgtgcta tgcaaaatag acatctttaa catagtcctg ttactatggt aacactttgc 300
tttctgaatt ggaagggaaa aaaaatgtag cgacagcatt ttaaggttct cagacctcca 360
gtgagt 366

<210> 203
<211> 409
<212> DNA
<213> Homo sapien

<400> 203
cgaggtactg aagaacccca tcatgtgaga gatcgctcaa agtcattaac acaaagcagt 60
gaaaatcatc cagcaaagca gtgctattat gagtgtgggc tatggaaaga cagcttttcc 120
tacactgata aagaaaaaaa aatgaggaaa ttatttcatc cccttgtgac atctgtgact 180
ttttggattt aataatcttg ctgtttttcc tctttatgac aaagaatata attgggagga 240
tgaagtgtct taaaaattgt agagaccagc tcactggaat gtttttccat ccctgtattc 300

atggcttgac tttgtgactg ctctacactg catgtctgac attgcagagt gagctatgtt 360
gaggtaaact ggttggttgc attattttgc aatcagcctg gtctctccc 409

<210> 204
<211> 440
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(440)
<223> n = A,T,C or G

<400> 204
acacacatcc tgatctagct atgtttatgt gtgttggggt gatggatgga caagaggtat 60
agttcaaatg agatcatttt tgtgaaatgg ctttgtaaac tgtaacatgc cctataaata 120
tgagattagc tttaatactg gccctgactc tccagtgtgg ctttgtgtgt ttgtctaaac 180
acttagttaa tatctgtcag tggtccattg cacaaggaac tgacacaatg gtatcctgtg 240
cctctgttgt tgttgttgtt gtttttttg cagttctaaa agcttagtta attgccttca 300
ttagcttaat atataccacg tgaaaagcat agaaaagcag aactcaaaac tcanagaata 360
aaggacagaa cataactaac tactgatgtg caccttagtt acctgatgca gggaattgaa 420
gcatataagc ttcatctagt 440

<210> 205
<211> 474
<212> DNA
<213> Homo sapien

<400> 205
acttgtccca tgctaggtaa caggaaaata atagtgattg ataagacata gtccctgtcc 60
tcaaagagtt aacagtctag caaggcagga actttgagaa aagaccaatg tgttcaaagg 120
aaaactcaca acctgggtct cccttctcag atggcacatt caagaaactg ttgcttatgc 180
ccctgggagc cagagcctta cttaagtctt accaagtcaa atatctatca gcctcagatg 240
atttgagcct ggtaaagtct tagcaataga tttgctgcct catgttccca tgaaaaccta 300
ataagagaga gccctttcaa ctcaggcata cggggggttt aaggataaca tgtttagtga 360
ccatgtggac attcagcaca ggtgagcttc tcaagtgaga gccatgtgtc cccaaaagaa 420
aggagggttt atccataaga ctttgctctc cctttcaaca ctgtggtggg aagt 474

<210> 206
<211> 344
<212> DNA
<213> Homo sapien

<400> 206
accgtccttc ttggggcaga tgtctgagat aaactgttcc acgcccccag ccaaaccaca 60
gcagttcaac gcatagtgga tggctttcag cgtttcccgc tggggctcat ccttggtttt 120
cagcttgttg taggtgtcct tgtaaaactc ctggacttcc ttaatcacct catccttgtg 180
ggaatatccc cagatggccg cagctatttc aatggcgaat atcaccaaga ggaagccgaa 240
gaacagtccc agcatgcact gggactcctg cacagccccg cagcagccca ggaagcccac 300
cagcatcatg agggcgccgg ctccgatcag aatatagact cctg 344

<210> 207
<211> 441
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature

<222> (1)...(441)
<223> n = A,T,C or G

<400> 207
acctcaattt ttccccccaat ttctggctac tactaaaagc cagaaagaac agaacagtgg 60
cctcaggaga tctgagtttg aatccttgct ctctaggatg caggtggctt gaagcagaat 120
gccacacctg caagttgatt agaactgcct ttcttcccag gcttgacata ggtattaagt 180
caaaattaca tgaaacccag tggtaaaaaa gcctctgaaa gctgtaacac cctcagtaat 240
aacaaaaggg atttttattt cacagctaaa gggaaaatag gtggagaagt taaaaaataa 300
tgtctgatcc tgttcctaag ttccaaacta tagccaacac tctgatgctg ctctttttct 360
tgtaggacca accgtcccag tttgcctggg actttctcat ttttacagag tcccaaatcc 420
tangaaactg gagcaactgg t 441

<210> 208
<211> 365
<212> DNA
<213> Homo sapien

<400> 208
ctggtgccag tgccagtgtc tgagccagtg ccagagccgg aacctgagcc agaacctgag 60
cctgttaaag aagaaaaact ttcgcctgag cctattttgg ttgatactgc ctctccaagc 120
ccaatggaaa catctggatg tgcccctgca gaagaagacc tgtgtcaggc tttctctgat 180
gtaattcttg cagtaaatga tgtggatgca gaagatggag ctgatccaaa cctttgtagt 240
gaatatgtga aagatattta tgcttatctg agacaacttg aggaagagca agcagtcaga 300
ccaaaatacc tactgggtcg ggaagtcact ggaaacatga gagccatcct aattgactgg 360
ctagt 365

<210> 209
<211> 191
<212> DNA
<213> Homo sapien

<400> 209
cgaggtacag aatataaagg agactgttga attcatacca tataaaactt gttaggtttt 60
taaacatagc aatcaaggct acaaaaacaa acctgtgttg tttttgtata gattgtaggt 120
ttatttttgg atttcatata catgactgaa ctgtgtgcaa ggcaatagtt agccttgatt 180
ttagcccaga g 191

<210> 210
<211> 373
<212> DNA
<213> Homo sapien

<400> 210
acttaattgt atatttcatt taaatagtcc ttctcagggg tttaataatt tagaatcaat 60
agttcccttc aaaacataat aaaatattta cactttataa aatattaacc cgattaacaa 120
tacagccgtg ttgtttataa gagtgtaact gaagtcctgc aaatcatgct gttgacacaa 180
gcctgtgagg ttagcgaagt gatccttagc aaaatgtaaa tgaagatctt cagacagtgg 240
tgtttataaa atagctcatt aatgacttag gattgaatcg ctccaaccat tcgcatcatc 300
agatataata atagtgacga atcagacagg aaagatcctg gctaaaccat ttgcattttt 360
ttccagaagt acc 373

<210> 211
<211> 336
<212> DNA
<213> Homo sapien

<400> 211

actgtaatct ttcttcatca aaatatgcaa aacagcatca tggattgtta agaaaaatat 60
tgagcttttc acttcaccat caaaaaattc ataccggtta agcttctcaa tgaagtcatc 120
atcagttcca acgatataca catctacctt gatcctgata aattcttgca aaatcgattt 180
aaggcccctc actgaagaaa catcaagaaa ggacactgct gaaaagtcga gaatgaggct 240
gtggaggctg attttgggga cctcaatgtt gagaggaaga tcatcattcc agtcaatgtg 300
gaaaggcagg tctgtggtat tgattgctgg tccagt 336

<210> 212
<211> 434
<212> DNA
<213> Homo sapien

<400> 212
accaccagca attttaagga aatcttcacc tgttgctttg taaacctcaa tataccgggt 60
ccccatgtga tgtttgtgcc tctgtagtgc taggtctcgg tgctcctcac ttacaaacct 120
aaccagagct tctccgttcc ttcgaccctg agcattcaga caaagtgctg cacctcccctt 180
ggcaatattg agtcctttga agaatcttgc aatatcttga tctgaagact gccatggtaa 240
acctcgtgcc ctgactacgg tgttatcatc aataagttcc atcttgctgc aagttccact 300
ttcaaacttg taattcactc tctctggatc tgaaaacctg tgattataag gctctgaaat 360
cattgctaaa attatattcc ccatatcttc aacttgagag gctccatatc gagagactga 420
actactcttc tcaa 434

<210> 213
<211> 515
<212> DNA
<213> Homo sapiens

<400> 213
actacacgac acgtactctt gaatacaagt ttctgatacc actgcactgt ctgagaattt 60
ccaaaacttt aatgaactaa ctgacagctt catgaaactg tcccaccaaga tcaagcagag 120
aaaataatta atttcatggg actaaatgaa ctaatgagga taatattttc ataatttttt 180
atttgaaatt ttgctgattc tttaaatgtc ttgtttccca gatttcagga aacttttttt 240
cttttaagct atccacagct tacagcaatt tgataaaata tacttttgtg aacaaaaatt 300
gagacattta cattttctcc ctatgtggtc gctccagact tgggaaacta ttcatgaata 360
tttatattgt atggtaatat agttattgca caagttcaat aaaaatctgc tctttgtatg 420
acagaataca tttgaaaaca ttggttatat taccaagact ttgactagaa tgtcgtattt 480
gaggatataa acccataggt aataaaccca caggt 515

<210> 214
<211> 353
<212> DNA
<213> Homo sapiens

<400> 214
acaagactca agtaaataga aaggcagctt tcaatcacaa atcagttttt cagattttac 60
tgtggaagca tatttaatgc acacatttga atgttacaca taaataattt taacgatgga 120
gtccaagttc tggattttac attagatctg catatataag acacttgtgg tcaaatttca 180
agattggtaa agccagtttc aagctgctta tattttgagt acctgcccgg gcggccgctaa 240
gggcgaattc tgcagatatc catcacactg gcggccgct cgagcatgca tctagagggc 300
ccaattcgcc ctatagtgag tcgtattaca attcactggc cgtcgtttta caa 353

<210> 215
<211> 699
<212> DNA
<213> Homo sapiens

<220>

<221> misc_feature
<222> (1)...(699)
<223> n=A,T,C or G

<400> 215
acacttgaaa ccaaatttct aaaacttgtt tttcttaaaa aatagttgtt gtaacattaa 60
accataacct aatcagtgtg ttcactatgc ttccacacta gccagtcttc tcacacttct 120
tctggtttca agtctcaagg cctgacagac agaagggctt ggagattttt tttctttaca 180
attcagtctt cagcaacttg agagctttct tcatgttgtc aagcaacaga gctgtatctg 240
caggttcgta agcatagaga cgatttgaat atcttccagt gatatcggct ctaactgtca 300
gagatgggtc aacaaacata atcctgggga catactggcc atcaggagaa aggtgtttgt 360
cagttgtttc ataaaccaga ttgaggagga caaactgctc tgccaatttc tggatttctt 420
tattttcagc aaacactttc tttaaagctt gactgtgtgg gcactcatcc aagtgatgaa 480
taatcatcaa gggtttgttg cttgtcttgg atttatatag agcttcttca tatgtctgag 540
tccagatgag ttggtcaccc caacctctgg agagggtctg gggcagtttg ggtcgagagt 600
cctttgtgtc ctttttggct ccaggtttga ctgtggtatc tctggccaga gtgtaggaga 660
nggccacaag gagcaagaat gctgacactg gaattttct 699

<210> 216
<211> 691
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<222> (1)...(691)
<223> n=A,T,C or G

<400> 216
ncgaggtaca ggtttcacta ttacaaatat atgatgttaa actaacaaac tcatgacctt 60
caaagatgtc ttcgtcccac gcacacacat ttgtaatttg tgtccatttg ctatttccct 120
tcttctataa tcttcaaatt atatagttat gcattgagtt ccctatgcat ctcacccatc 180
tcctttatct cagccttctc atactttgcc attctcttct ttctggaaat aaccagcaca 240
acaattccag caacaactgc tatcaccaca accacaataa cagcaataac accagctttt 300
agaccctgca ttgagaattc aggtgctttt tcatcaacat aataaattaa agtttgacca 360
ggatccagat ccagttgttc cccatttact gtcaggtcca ttttcttaga atgaaacaag 420
gattcacctt taacatcttt ttcaaaataa taagccacat cagctatgtc cacatcattc 480
tgagtttttt gagaagaatt ttgaaccaga tcaatagtga taacattatt ctcatacaaa 540
atactcgtga taaatttggg atccagttga taacgcgttg tgatctcctt ctgaagtgca 600
gtccgcaaac ttttactatc ataagggttt tctcttgctt tgnggtttag ttcaatggat 660
gatccagtag ggtctcactc gctcagagca a 691

<210> 217
<211> 497
<212> DNA
<213> Homo sapiens

<400> 217
ctgtgctcct ggatggtttt accacaagtc caattgctat ggttacttca ggaagctgag 60
gaactggtct gatgccgagc tcgagtgtca gtcttacgga aacggagccc acctggcatc 120
tatcctgagt ttaaaggaag ccagcaccat agcagagtac ataagtggct atcagagaag 180
ccagccgata tggattggcc tgcacgaccc acagaagagg cagcagtggc agtggattga 240
tggggccatg tatctgtaca gatcctggtc tggcaagtcc atgggtggga acaagcactg 300
tgctgagatg agctccaata acaacttttt aacttggagc agcaacgaat gcaacaagcg 360
ccaacacttc ctgtgcaagt accgaccata gagcaagaat caagattctg ctaactcctg 420
cacagccccg tcctcttcct ttctgctagc ctggctaaat ctgctcatta tttcagaggg 480
gaaacctagc aaactaa 497

<210> 218
<211> 603
<212> DNA
<213> Homo sapiens

<400> 218
acaaaggcga aagagtggat ggcaaccgtc aaattgtagg atatgcaata ggaactcaac 60
aagctacccc agggcccgca tacagtggtc gagagataat ataccccaat gcatccctgc 120
tgatccagaa cgtcacccag aatgacacag gattctacac cctacacgtc ataaagtcag 180
atcttgtgaa tgaagaagca actggccagt tccgggtata cccggagctg cccaagccct 240
ccatctccag caacaactcc aaacccgtgg aggacaagga tgctgtggcc ttcacctgtg 300
aacctgagac tcaggacgca acctacctgt ggtgggtaaa caatcagagc ctcccggtca 360
gtcccaggct gcagctgtcc aatggcaaca ggaccctcac tctattcaat gtcacaagaa 420
atgacacagc aagctacaaa tgtgaaaccc agaacccagt gagtgccagg cgcagtgatt 480
cagtcatcct gaatgtcctc tatggcccgg atgcccccac catttcccct ctaaacacat 540
cttacagatc aggggaaaat ctgaacctct cctgccacgc agcctctaac ccacctgcac 600
agt 603

<210> 219
<211> 409
<212> DNA
<213> Homo sapiens

<400> 219
ctgagagacc aggagaagtt ccagatgcag agactgtgat gctcttgact atggaattat 60
tgcggccagt agccaagtta gagacaaaac aggcgtaggt cccgttatta tttggcgtga 120
ttttggcgat aaagagaact tgtgtgtgtt gctgcggtat cccattgata cgccaagaat 180
actgcgggga tgggttagag gccgagtggc aggagaggtt gaggttcgct cccgaaaggt 240
aagacgagtc tggggggg aa atgatggggg tgtccggccc atagaggaca tccagggtga 300
ctgggtcact gcggtttgca ctcactgagt tctggattcc acatacatag gctcttgcgt 360
catttcttgt gacattgaat agagtgaggg tcctgttgcc attggacag 409

<210> 220
<211> 635
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<222> (1)...(635)
<223> n=A,T,C or G

<400> 220
acagtgatag ctccccctgg gcaatacaat acaagaacag tgggttttgt caaattggaa 60
caaggaaaca gaaccacaga aataaataca ttggttaaca tcagattagt tcaggttact 120
tttttgtaaa agttaaagta gaggggactt ctgtattatg ctaactcaag tagactggaa 180
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ttttanagaa tacactacac cagggagtat gactactagt atgactatta ggagggtaat 540
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tgagtcagat gagtgtagcc attttaacca agcag 635

<210> 221
<211> 484
<212> DNA

<213> Homo sapiens

<400> 221
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actcgccggg ctggcaacct ggaacaaaag tcctgatcca gtagtcacac ttctttttcc 180
taaacaggac ggaggtgaca ttgtagctct tgtcttcttt cagctcatag atggtggcat 240
acatcttttg cggtctttg tcttctctga gaattgcatt ccctgccagg cctaccacat 300
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tgtg 484

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<211> 566
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<222> (1)...(566)
<223> n=A,T,C or G

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tcctctctaa taactttga tagacagggg ctagtcgcac agacctctgg gaagccctgg 180
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cctgaggtct gttggctgga ggctgcagaa cattggtgat gacatggacc acgccatttg 300
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cgctaaccag gatttcatca ccaatgtggt atttcaggat gttggcaagt tccttggcat 480
ctcccaagag tctgctccgt tctcttggtg gcagggctcg gaaggcttca tttgtgggag 540
caaagactgt gtagacttcc tttccc 566

<210> 223
<211> 478
<212> DNA
<213> Homo sapiens

<400> 223
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tatttatgct gaattgtgat ttttttatgc caaatttttt ttagttctaa tcattgatga 120
tagcttggaa ataaataatt atgccatggc atttgacagt tcattattcc tataagaatt 180
aaattgagtt tagagagaat ggtggtgttg agctgattat taacagttac tgaaatcaaa 240
tatttatttg ttacattatt ccatttgtat tttaggtttc cttttacatt ctttttatat 300
gcattctgac attacatatt ttttaagact atggaaataa tttaaagatt taagctctgg 360
tggatgatta tctgctaagt aagtctgaaa atgtaatatt ttgataatac tgtaatatac 420
ctgtcacaca aatgcttttc taatgtttta accttgagta ttgcagttgc tgctttgt 478

<210> 224
<211> 323
<212> DNA
<213> Homo sapiens

<400> 224
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ttcaatcaat caacttcatc ggaggccagc ccctccggcc ccagggaccc ccgatgatgc 120

caccttgccc taccatggaa ggacccccaa ccttcaaccc gcctgtgcca tatttcggga 180
ggctgcaagg agggctcaca gctcgaagaa ccatcatcat caagggctat gtgcctccca 240
caggcaagag ctttgctatc aacttcaagg tgggctcctc aggggacata gctctgcaca 300
ttaatccccg catgggcaac ggt 323

<210> 225
<211> 147
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<222> (1)...(147)
<223> n=A,T,C or G

<400> 225
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ccaaataata naattgctcc ctaccagctg aacagggagg agtctgtgca gttnctgaca 120
cttgttgttg aacatggtta aatacaa 147

<210> 226
<211> 104
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<222> (1)...(104)
<223> n=A,T,C or G

<400> 226
nncaggnaca tgtgtgaaaa caatattgta tactaccata gtgagccatg antntntaaa 60
aaaaaaataa atgttttggg ggngatntgt attctccaac ttgg 104

<210> 227
<211> 491
<212> DNA
<213> Homo sapiens

<400> 227
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ttattatgtt ttatcattaa ttattcaata aatttttatt taaaaagtcg ccctacttag 120
aaatcttctg tgggggtggg agggacaaaa gattacaaac caaaactcag gagatggtaa 180
cactggaatt gataaaatca cctgggatta gtcgtataac tctgaaccac caaacctctg 240
ctatcaagcc ttgctacagt catggctgtc cagaaagatt tacagttatt tttctgagaa 300
aggatccatg ggctttaaga acttcagaac tttaagaact tcagaagttc ttaagttgct 360
gaagctcaag taacgaagtt gaatgcaatc aaaaaaagaa taccagggag tcaaggcttg 420
agaggcacat tcttatccta aagtgactgc tcaaacctga cgagaccaag taaattactg 480
aagatacaaa g 491

<210> 228
<211> 328
<212> DNA
<213> Homo sapiens

<400> 228
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tgggatttcc attgatgaca agcttcccgt tctcagcctt gacggtgcca tggaatttgc 120

catgggtgga atcatattgg aacatgtaaa ccatgtagtt gaggtcaatg aaggggtcat 180
tgatggcaac aatatccact ttaccagagt taaaagcagc cctggtgacc aggcgcccaa 240
tacgaccaaa tccgttgact ccgaccttca ccttccccat ggtgtctgag cgatgtggct 300
cggctggcga cgcaaaagaa gatgcggc 328

<210> 229
<211> 689
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<222> (1)...(689)
<223> n=A,T,C or G

<400> 229
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cagggagaat ctaccacctt ccagagctgg ccaaactcga aggacactac ccctgcacct 240
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agcacaacac acacaatatc ttcagctcct agcaccacat ctgcccttgt tgaagaacct 600
accagctacc acagcagccc gggctcaact gcaacaacac acttcccttg acaggttcca 660
caacctcaag gccgtagtgg agggaaatc 689

<210> 230
<211> 483
<212> DNA
<213> Homo sapiens

<400> 230
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ctcaggcctg aggtgtgtgc cactcagaga cttcacctaa ctagagacag gcaaactgca 180
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taacttaaca gagtgtcaga tctatcttgt caatcccaac gttttacata aaataagaga 420
tcctttagtg cacccagtga ctgacattag cagcatcttt aacacagccg tgtgttcaaa 480
tgt 483

<210> 231
<211> 447
<212> DNA
<213> Homo sapiens

<400> 231
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aaactaagtg cccatcaaag atttgtttgg tataaataaa gaattatttg ttttgttttc 300
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aattggctat gttgttccaa tgaatgt 447

<210> 232
<211> 649
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<222> (1)...(649)
<223> n=A,T,C or G

<400> 232
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tggacatgaa gattggggtg taacacctac accattatct tggaaagaaa caaccgttgg 240
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cattgcgaat ctttttagc ataaaatttt ctattctttt tgttttttgt gttttgttct 360
ttaaagtcag gtccaatttg taaaaacagc attgctttct gaaattaggg cccaattaat 420
aatcagcaag aatttgatcg ttccagttcc cacttggagg cctttcatcc ctcgggtgtg 480
ctatggatgg cttctaacaa aaactacaca tatgtattcc tgatcgccaa cctttccccc 540
accagctaag gacatttccc agggttaata gggcctggtc cctgggagga aatttgaatg 600
ggtccatttt gcccttncat agcctaatcc ctgggcattg ctttncact 649

<210> 233
<211> 396
<212> DNA
<213> Homo sapiens

<400> 233
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atttagtgat tctgaatggc tactctgcct gccagt 396

<210> 234
<211> 4627
<212> DNA
<213> Homo sapiens

<400> 234
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<210> 235
<211> 169
<212> PRT
<213> Homo sapiens

<400> 235

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Ala Leu Leu Leu Cys Leu Gly Phe His Leu Leu Gln Ala Val Leu Ser
            20                  25                  30

Thr Thr Val Ile Pro Ser Cys Ile Pro Gly Glu Ser Ser Asp Asn Cys
        35                  40                  45

Thr Ala Leu Val Gln Thr Glu Asp Asn Pro Arg Val Ala Gln Val Ser
    50                  55                  60

Ile Thr Lys Cys Ser Ser Asp Met Asn Gly Tyr Cys Leu His Gly Gln
65                  70                  75                  80

Cys Ile Tyr Leu Val Asp Met Ser Gln Asn Tyr Cys Arg Cys Glu Val
                85                  90                  95

Gly Tyr Thr Gly Val Arg Cys Glu His Phe Phe Leu Thr Val His Gln
            100                 105                 110

Pro Leu Ser Lys Glu Tyr Val Ala Leu Thr Val Ile Leu Ile Ile Leu
        115                 120                 125

Phe Leu Ile Thr Val Val Gly Ser Thr Tyr Tyr Phe Cys Arg Trp Tyr
    130                 135                 140

Arg Asn Arg Lys Ser Lys Glu Pro Lys Lys Glu Tyr Glu Arg Val Thr
145                 150                 155                 160

Ser Gly Asp Pro Glu Leu Pro Gln Val
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<210> 236
<211> 894
<212> DNA
<213> Homo sapiens

<400> 236

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cagggattcg ccattccgat cgggcaggcg atggcgatcg cgggccagat caagcttccc     120
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<210> 237
<211> 297
<212> PRT
<213> Homo sapiens

<400> 237
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            20                  25                  30
Ile Ala Gly Gln Ile Lys Leu Pro Thr Val His Ile Gly Pro Thr Ala
        35                  40                  45
Phe Leu Gly Leu Gly Val Val Asp Asn Asn Gly Asn Gly Ala Arg Val
    50                  55                  60
Gln Arg Val Val Gly Ser Ala Pro Ala Ala Ser Leu Gly Ile Ser Thr
65                  70                  75                  80
Gly Asp Val Ile Thr Ala Val Asp Gly Ala Pro Ile Asn Ser Ala Thr
                85                  90                  95
Ala Met Ala Asp Ala Leu Asn Gly His His Pro Gly Asp Val Ile Ser
            100                 105                 110
Val Thr Trp Gln Thr Lys Ser Gly Gly Thr Arg Thr Gly Asn Val Thr
        115                 120                 125
Leu Ala Glu Gly Pro Pro Ala Glu Phe Asp Ala Phe Leu Lys Tyr Glu
    130                 135                 140
Lys Ala Asp Lys Tyr Tyr Tyr Thr Arg Lys Cys Arg Asn Leu Leu Ser
145                 150                 155                 160
Phe Leu Arg Gly Thr Cys Ser Phe Cys Ser Arg Thr Leu Arg Lys Gln
                165                 170                 175
Leu Asp His Asn Leu Thr Phe His Lys Leu Val Ala Tyr Met Ile Cys
            180                 185                 190
Leu His Thr Ala Ile His Ile Ile Ala His Leu Phe Asn Phe Asp Cys
        195                 200                 205
Tyr Ser Arg Ser Arg Gln Ala Thr Asp Gly Ser Leu Ala Ser Ile Leu
    210                 215                 220
Ser Ser Leu Ser His Asp Glu Lys Lys Gly Gly Ser Trp Leu Asn Pro
225                 230                 235                 240
Ile Gln Ser Arg Asn Thr Thr Val Glu Tyr Val Thr Phe Thr Ser Arg
                245                 250                 255
Gly Gln Thr Glu Glu Ser Met Asn Glu Ser His Pro Arg Lys Cys Ala
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<210> 243
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<212> DNA
<213> Artificial Sequence

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<223> PCR primer

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gaagggctcc aaaccacctc ttgacaatgg gaaactgggt ggttaaccac tggttttcag 240
ttttgtttct ggttgtttgg ttagggctga atgttttcct gtttgtggat gccttcctga 300
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tcttcctcac cggatgggac agcaatattg ttggtcatgc agcattaaac tttgacaagg 1680
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ataattaatg ataaaacata ataataaaca taaataataa acaaaattac cgagaacccc 2460
atccccatat aacaccaaca gtgtacatgt ttactgtcac ttttgatatg gtttatccag 2520
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<212> PRT
<213> Homo sapiens

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20 25 30
Tyr Glu Lys Ala Asp Lys Tyr Tyr Tyr Thr Arg Lys Ile Leu Gly Ser
35 40 45
Thr Leu Ala Cys Ala Arg Ala Ser Ala Leu Cys Leu Asn Phe Asn Ser
50 55 60
Thr Leu Ile Leu Leu Pro Val Cys Arg Asn Leu Leu Ser Phe Leu Arg
65 70 75 80
Gly Thr Cys Ser Phe Cys Ser Arg Thr Leu Arg Lys Gln Leu Asp His
85 90 95
Asn Leu Thr Phe His Lys Leu Val Ala Tyr Met Ile Cys Leu His Thr
100 105 110
Ala Ile His Ile Ile Ala His Leu Phe Asn Phe Asp Cys Tyr Ser Arg
115 120 125
Ser Arg Gln Ala Thr Asp Gly Ser Leu Ala Ser Ile Leu Ser Ser Leu
130 135 140
Ser His Asp Glu Lys Lys Gly Gly Ser Trp Leu Asn Pro Ile Gln Ser
145 150 155 160
Arg Asn Thr Thr Val Glu Tyr Val Thr Phe Thr Ser Val Ala Gly Leu
165 170 175
Thr Gly Val Ile Met Thr Ile Ala Leu Ile Leu Met Val Thr Ser Ala
180 185 190
Thr Glu Phe Ile Arg Arg Ser Tyr Phe Glu Val Phe Trp Tyr Thr His
195 200 205
His Leu Phe Ile Phe Tyr Ile Leu Gly Leu Gly Ile His Gly Ile Gly
210 215 220
Gly Ile Val Arg Gly Gln Thr Glu Glu Ser Met Asn Glu Ser His Pro
225 230 235 240
Arg Lys Cys Ala Glu Ser Phe Glu Met Trp Asp Asp Arg Asp Ser His
245 250 255
Cys Arg Arg Pro Lys Phe Glu Gly His Pro Pro Glu Ser Trp Lys Trp
260 265 270
Ile Leu Ala Pro Val Ile Leu Tyr Ile Cys Glu Arg Ile Leu Arg Phe
275 280 285
Tyr Arg Ser Gln Gln Lys Val Val Ile Thr Lys Val Val Met His Pro
290 295 300
Ser Lys Val Leu Glu Leu Gln Met Asn Lys Arg Gly Phe Ser Met Glu
305 310 315 320
Val Gly Gln Tyr Ile Phe Val Asn Cys Pro Ser Ile Ser Leu Leu Glu
325 330 335
Trp His Pro Phe Thr Leu Thr Ser Ala Pro Glu Glu Asp Phe Phe Ser
340 345 350
Ile His Ile Arg Ala Ala Gly Asp Trp Thr Glu Asn Leu Ile Arg Ala

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Phe Glu Gln Gln Tyr Ser Pro Ile Pro Arg Ile Glu Val Asp Gly Pro
    370                 375                 380
Phe Gly Thr Ala Ser Glu Asp Val Phe Gln Tyr Glu Val Ala Val Leu
385                 390                 395                 400
Val Gly Ala Gly Ile Gly Val Thr Pro Phe Ala Ser Ile Leu Lys Ser
                405                 410                 415
Ile Trp Tyr Lys Phe Gln Cys Ala Asp His Asn Leu Lys Thr Lys Lys
            420                 425                 430
Ile Tyr Phe Tyr Trp Ile Cys Arg Glu Thr Gly Ala Phe Ser Trp Phe
        435                 440                 445
Asn Asn Leu Leu Thr Ser Leu Glu Gln Glu Met Glu Glu Leu Gly Lys
    450                 455                 460
Val Gly Phe Leu Asn Tyr Arg Leu Phe Leu Thr Gly Trp Asp Ser Asn
465                 470                 475                 480
Ile Val Gly His Ala Ala Leu Asn Phe Asp Lys Ala Thr Asp Ile Val
                485                 490                 495
Thr Gly Leu Lys Gln Lys Thr Ser Phe Gly Arg Pro Met Trp Asp Asn
            500                 505                 510
Glu Phe Ser Thr Ile Ala Thr Ser His Pro Lys Ser Val Val Gly Val
        515                 520                 525
Phe Leu Cys Gly Pro Arg Thr Leu Ala Lys Ser Leu Arg Lys Cys Cys
    530                 535                 540
His Arg Tyr Ser Ser Leu Asp Pro Arg Lys Val Gln Phe Tyr Phe Asn
545                 550                 555                 560
Lys Glu Asn Phe
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